RECENT ADVANCES IN CHEMOTHERAPY

By

G. M. FINDLAY
C.B.E., M.D., D.Sc.
Wellcome Bureau of Scientific Research, London

With a Foreword by

C. M. WENYON
C.M.G., *C.B.E.*, M.B., B.S., F.R.S.
Director-in-Chief of the Wellcome Bureau
of Scientific Research, London

SECOND EDITION

LONDON
J. & A. CHURCHILL LTD.
104 GLOUCESTER PLACE
PORTMAN SQUARE
1939

To
M. F.

First published . 1930
Second Edition . 1939

Printed in Great Britain

PREFACE TO THE SECOND EDITION

THE eight years which have elapsed since the first appearance of this book have witnessed a discovery equal to, if not more important than, the original introduction of arsphenamine by Ehrlich. In the sulphonamide drugs have been found chemotherapeutic agents that are revolutionizing the treatment of acute bacterial infections. So great, in fact, has been the stimulus to research that, from being a subject of interest to the few, chemotherapeutic investigations are now being actively pursued throughout the world. In view of the number of papers appearing there is danger that any book which attempts to summarize them will be out of date almost before it is published.

Although the discovery of remedies against bacterial infections naturally overshadows all others, numerous other advances have to be chronicled. Atebrin, for instance, has taken an important place in the chemotherapy of malaria, new trypanocidal compounds have been produced, while much of fundamental importance has been learnt of the action of drugs in sleeping sickness.

To find space for these and other advances this second edition has been almost entirely rewritten. Much of interest has inevitably had to be discarded, but this has been done with an easy conscience because of the publication by V. Fischl and H. Schlossberger of a "Handbuch der Chemotherapie" (1932, English translation 1933), a work which satisfies the long-felt need for a text-book of chemotherapy.

Mention should also be made of monographs such as "Advances in the Therapeutics of Antimony" (1938), first published in German in 1937 under the title "Ergebnisse und Fortschritte der Antimontherapie," by Hans Schmidt and F. M. Peter; "Die Methoden der experimentellen Chemotherapie" (1938), by

C. Schilling; "Chaulmoograöl" (1938), by H. Schlossberger; and "Sulfanilamide Therapy of Bacterial Infections" (1938), by R. R. Mellon, P. Gross and F. B. Cooper.

In the preparation of this edition my thanks are again due to Dr. C. M. Wenyon, Director-in-Chief of the Wellcome Research Institution for his interest and advice. My sincere thanks are also due to Dr. T. A. Henry, Director of the Wellcome Chemical Research Laboratories, and his staff, Messrs. W. H. Gray, H. Paget, T. M. Sharp and W. Solomon, who have generously placed at my disposal their specialized knowledge. I owe also a debt of gratitude for much information and assistance to Dr. G. A. H. Buttle and Mr. A. B. MacIntyre of the Wellcome Physiological Research Laboratories, Dr. F. M. Peter of the I.G. FarbenIndustrie and my colleagues Dr. J. C. Broom, Dr. R. D. Mackenzie, Dr. F. O. MacCallum and Dr. F. Murgatroyd. The untiring help of Miss I. M. Bellis, Librarian to the Wellcome Bureau of Scientific Research, has again been invaluable, while Mr. E. A. Boulter has kindly assisted in the preparation of the Index. I am indebted to Dr. Helmholz of the Mayo Clinic for permission to reproduce the graph on p. 476, while to my publishers I must again express my thanks for their courtesy and long-suffering.

G. M. FINDLAY.

FOREWORD TO THE FIRST EDITION

THE work of Ehrlich and those that immediately followed him opened up new fields of inquiry in which the old empiricism was replaced by definite attempts to correlate the specific action of drugs on parasites with chemical constitution. An ever-increasing number of investigators have studied the action of drugs on parasites, and by varying the constitution have found that the action is thereby increased, diminished or not altered at all. The literature of the subject, which is of the greatest importance as regards improvements in therapeutics, is now so extensive, that without some adequate guide it is difficult, if not impossible, for an individual to grasp its significance. With the exception of "The Principles and Practice of Chemotherapy," by J. A. Kolmer, a work which deals more especially with the treatment of syphilis, and scarcely at all with many other important branches of chemotherapy, there is no work available in the English language which describes the present position of the subject from the point of view of medicine. On this account Dr. G. M. Findlay's survey of the recent advances should prove of great service to the many workers in this field.

The greater part of the work is necessarily devoted to a study of the action of chemical agents on diseases due to helminths, protozoa and spirochætes, for as yet little progress has been made in the chemotherapeutic treatment of bacterial diseases, and still less in those of virus infections. The reason for this is to be found in the fact that helminths, protozoa and spirochætes can readily be detected by direct examination of, for instance, a blood film or fæces. Their presence before and their absence after the administration of a drug is relatively easy to determine, whereas the detection of bacteria and ultra-microscopic viruses requires more elaborate cultural or inoculative tests. Furthermore, protozoal infections, such as those due to trypanosomes in experimental animals, run fairly regular and rapid courses, during which the parasites are easily seen in the blood. It is in these cases a relatively simple and rapid procedure to determine whether the infection is influenced in any way by chemotherapeutic agents. On this account a large proportion of the published works deal with protozoal infections, particularly those due to trypanosomes in experimental animals. Quite apart from the reasons just mentioned, the study of chemotherapy has developed along the lines of testing drugs on parasites of an animal nature, for it has been found that they are actually more vulnerable to drugs introduced into the body of the host than bacteria and allied organisms. It might be true to state that the more highly organised a parasite is, the more probable it is that it will be influenced by chemotherapeutic agents.

C. M. WENYON.

PREFACE TO THE FIRST EDITION

Recent advances in such a subject as chemotherapy are by no means easily defined. Countless drugs have been introduced for the specific treatment of parasitic diseases, but it is only after constant trials that the value of any compound can be accurately assessed and by this time its introduction can hardly be described as recent. Nevertheless, when such a period as the last ten years is surveyed as a whole, it is apparent that certain definite advances have been made in chemotherapeutic treatment, while in addition to the use of new compounds, a better understanding has been gained of the mode of action of well-established medicaments.

An attempt has therefore been made to describe the more important advances in chemotherapy which have occurred during the post-war period, special emphasis being laid on the fact that the focus of attention has tended to shift from a study of the direct interaction of drugs with infecting organisms to an investigation of the part played by the tissues in chemotherapeutic treatment.

This book could not have been written without the invaluable assistance, which I most gratefully acknowledge, of Dr. C. M. Wenyon, Director-in-Chief of the Wellcome Bureau of Scientific Research, and of Dr. T. A. Henry, Director of the Wellcome Chemical Research Laboratories.

My sincere thanks are also due to Mr. W. H. Gray, Dr. J. W. Trevan, and my colleagues Major H. C. Brown and Dr. J. C. Broom for their kindness in reading proofs and for their constant aid and advice. The help of Miss I. M. Bellis, Librarian to the Wellcome Bureau of Scientific Research, has also been of the utmost value. I am indebted to Colonel R. B. Lloyd, I.M.S., and the Editor of the *Indian Journal of Medical Research* for permission to reproduce the graphs illustrating the chapter on kala azar, and to the Wellcome Historical Museum for the use of the portraits of Ehrlich, Pelletier, Caventou and Magendie. Finally, I must express to my publishers my appreciation of their courtesy and ever-ready help.

G. M. FINDLAY.

CONTENTS

CHAP. PAGE

PREFACE TO SECOND EDITION v

FOREWORD TO FIRST EDITION vii

PREFACE TO FIRST EDITION ix

I. CHEMOTHERAPY OF HELMINTHIC INFECTIONS . 1

ANKYLOSTOMIASIS 25

SCHISTOSOMIASIS 43

II. CHEMOTHERAPY OF AMŒBIASIS 58

III. CHEMOTHERAPY OF LEISHMANIASIS . . . 87

KALA AZAR 87

CUTANEOUS AND MUCO-CUTANEOUS LEISHMANIASIS 101

IV. CHEMOTHERAPY OF MALARIA 106

BLACKWATER FEVER 152

V. CHEMOTHERAPY OF TRYPANOSOMIASIS . . . 155

VI. CHEMOTHERAPY OF SYPHILIS 238

VII. CHEMOTHERAPY OF TUBERCULOSIS . . . 287

VIII. CHEMOTHERAPY OF LEPROSY 298

IX. CHEMOTHERAPY OF ACUTE BACTERIAL INFECTIONS 322

STREPTOCOCCAL INFECTIONS 345

PNEUMOCOCCAL INFECTIONS 383

MENINGOCOCCAL INFECTIONS 392

GONOCOCCAL INFECTIONS 398

STAPHYLOCOCCAL INFECTIONS 409

BRUCELLA INFECTIONS 413

INFECTIONS DUE TO *BACTERIUM COLI* . . 415

X. CHEMOTHERAPY OF VIRUS INFECTIONS . . . 486

AUTHOR INDEX 496

SUBJECT INDEX 507

RECENT ADVANCES IN CHEMOTHERAPY

CHAPTER I

THE CHEMOTHERAPY OF HELMINTHIC INFECTIONS

THE most primitive chemotherapy of which there is record was undoubtedly concerned with the finding of remedies for the intestinal worms, which form an example of parasitism so obvious that their presence in the fæces cannot have failed to attract attention at a very early stage of man's history. Though the relationship between cause and effect is notoriously difficult to determine, it is probable that in the course of time the evacuation of these worms came to be associated with the eating of certain substances and, since vegetables form a large part of the food supply of primitive man, it is not surprising that the earliest known anthelmintics were also of vegetable origin. It is, in fact, only comparatively recently that organic synthetic compounds have come into use as anthelmintics. Among the many hundreds of vegetable substances formerly used in this way some are now of historical interest only; others, such as male fern, which was esteemed by Theophrastus (370–285 B.C.) and Galen (A.D. 130–201), still retain their old prestige.

Hall (1928) divides the study of anthelmintics into three periods. The first of these was characterised by the purest empiricism, when any drug which would remove some worms was recommended, without consideration of the number of worms that remained behind. The use of many newer anthelmintics, it may be added, hardly rests on a firmer basis. The first epoch of purely empirical medication came to an end with a recognition of the economic importance of hookworm disease. In 1880, male fern was used by Perroncito for the treatment of hookworm disease, thymol by Bozzolo in 1881.

In the same year Baumler reported unfavourably on the efficiency of oil of chenopodium, a remedy originally used by the Aztecs. Thirty years had to elapse before Schüffner and Verwoort (1913) demonstrated its real value. Meanwhile Bentley (1904) had found β-naphthol satisfactory and, with a growing realisation of the good results obtained by thymol, male fern passed out of fashion for the treatment of ankylostomiasis.

This second period of critical empiricism was characterised by a growing tendency to test the absolute and relative efficiency of the various anthelmintics used for the removal of hookworms. The evacuation of worms and the clinical improvement were naturally the first criteria used in formulating judgments on drugs. Unfortunately, the numbers of worms removed tell nothing of the numbers that remain and clinical improvement cannot be accurately measured.

Efforts were therefore made to devise egg-counting methods and of these two the Stoll technique (*cf.* Stoll and Hausheer, 1926) and Lane's direct centrifugal floatation method (D.C.F.) have been largely used. Full details of technique are given by Lane (1932). It must be remembered that even in the hands of skilled technicians egg-counts do not measure the actual number of worms in the intestine, although Lane (1935) claims that by direct centrifugal floatation pushed to finality (D.C.F.F.) it is possible to detect a single normally ovi-positing hookworm. Maplestone and Mukerji (1933), however, have found that certain anthelmintics may temporarily inhibit the egg-laying capacity of worms, full activity being recovered after a longer or shorter period.

More accurate evidence of anthelmintic efficiency was obtained by using a more or less standard treatment following a test treatment, the worms removed on both occasions being carefully identified and counted. This method, which can be applied to man, has given much information, but it lacks exactness if the relative efficiency of various anthelmintics is to be compared, since it presupposes that the number of worms harboured at the beginning of each experiment is approximately the same.

The third epoch dates from the year 1915 when, in the United States, Hall and his colleagues introduced a critical method for testing anthelmintics. This was not the first time that critical testing had been attempted, for Perroncito (1880) and Grassi and Calandruccio (1884) had already employed a method of critical testing in their studies on liver fluke disease of sheep, in which they had established by post-mortem examination that male fern can kill liver flukes. Both as a practical proposition and as a scientific procedure their work remained neglected for a third of a century.

The method of critical testing which Hall adopted in studying anthelmintics was to administer known doses of drugs to animals of various species, to collect all the worms from the fæces for at least four days, sometimes for weeks or months, to kill the animals and at autopsy to collect, identify and count all the worms present. This method gives information as to the number of worms originally present, the number removed and the number left after treatment, though some worms that have been killed but not expelled may have been digested in the intestine. It also enables new drugs to be tested which it would be inadvisable to administer to man without preliminary tests on animals.

Before figures of sufficient accuracy for use in comparing the efficiency of two drugs can be obtained by this method of critical testing it is necessary to ensure certain essential experimental conditions. Thus all the animals must have been exposed to a similar risk of infection and must be equally susceptible to infestation by the particular helminth under investigation. All the parasites harboured by an individual must be equally exposed and equally susceptible to the action of the drug that is to be tested.

Seddon and Ross (1928) and Roberts (1932), in order to obtain a mathematical comparison of the efficiency of various anthelmintics, employed a slightly different method of critical testing. They first determined the mean number (X) of the specific helminth that was found to be harboured by a random sample of hosts of similar age and reputedly maintained in an environment in which all individuals were exposed to a similar risk of infection. From the same population of hosts another sample was treated with the drug to be investigated and the mean number (Y) of the specific helminth remaining at autopsy in the treated animals was now determined. The efficiency (E) of the drug was then expressed by the formula :—

$$E = \frac{100\,(X - Y)}{X}$$

Carr-Fraser (1935) has, however, pointed out that this bald figure does not take into consideration the variation in the degree of infestation found to exist in the untreated animals. A more reliable estimate is probably obtained by comparing the residual infestation of each of the treated hosts with the initial infestation of each of the untreated hosts, for which purpose the derivation and use of equations are given. The application of these equations by Carr-Fraser to the results obtained by Seddon and Ross and Roberts for the efficiency of carbon tetrachloride and tetrachloroethylene in sheep and lambs against *Hæmonchus contortus* produced interesting results. Thus, it was deduced from Roberts's results that tetrachloroethylene at a dose rate of 5 c.c. was in lambs just significantly more efficient than carbon tetrachloride at a dose rate of 1 c.c., but in adult sheep carbon tetrachloride at a dose rate of 5 c.c. was more efficient than the same dose of tetrachloroethylene : in the hands of Seddon and Ross there was no significant difference in the efficiency of the two drugs.

A further factor that must be taken into account in comparing the efficiency of different anthelmintics is that closely allied species such as *Necator americanus* and *Ancylostoma duodenale* are not equally susceptible to a drug such as hexylresorcinol. It is therefore essential that in comparing two investigations all the experimental conditions should be similar, otherwise no *ex cathedra* statement can be made for or against any differences in the results obtained on different occasions by different observers.

Difficulties in assessing the value of anthelmintics account for the fact that even to-day Lamson and Ward (1932) can give a list of nearly two hundred substances reputed to have an anthelmintic action.

In the case of many anthelmintics there is little or no evidence as to whether or not the parasites are directly attacked by the drug. So far as their actions on intestinal worms are concerned Lamson and Ward (1932) divide anthelmintics into five groups according to whether they cause :—

(1) Temporary narcosis or paralysis with recovery.
(2) Narcosis or paralysis followed by death.
(3) Injury to the cuticle and death.
(4) Digestion of the worms.
(5) Unexplained death.

There are still many unsolved problems in the chemotherapy of helminthic infections. The ideal anthelmintic for any type of infestation has not yet been obtained. In the treatment of filariasis only a very feeble beginning has as yet been made : in the treatment of cysticercosis, hydatids and dracontiasis, with the doubtful exception of acriflavine, no drugs are available.

These failures are due in large measure both to the lack of suitable experimental animals in which the infections can be maintained, and to the absence of *in vitro* methods of testing anthelmintics such as that developed by Lamson and Brown (1936) for the study of ascaricides. The irrationality of using animals such as leeches or earthworms for evaluating the activity of anthelmintics destined for use in human intestinal infections cannot be over-emphasized. Lamson and Ward (1936) have recently pointed out that a comparative study of the lethality *in vitro* of 121 widely diversified chemical substances on earth worms and on pig *Ascaris* showed that in only 6 per cent. of cases was there any evidence of correlation.

References

BAUMLER, C., 1881, Ueber die Abtreibung des *Anchylostomum duodenale. KorrespBl. schweiz. Ärz.*, XI., 481.

BENTLEY, C. A., 1904, Some notes on ankylostomiasis in Assam. *Indian med. Gaz.*, XXXIX., 135.

BOZZOLO, C., 1881, Ueber die Anwendung der Thymosäure als Wurmmittel in der Anchylostomen-Anæmie. *Zbl. klin. Med.*, **II.**, 1.

CARR-FRASER, W. A., 1935, The comparison of the efficiency of anthelmintics. *Parasitology*, **XXVII.**, 465.

GRASSI, G. B., and CALANDRUCCIO, F., 1884, Intorno ad una malattia parassitaria. *Agric. calabro-sic.*, *Girgenti*, **IX.**

HALL, M. C., 1928, A theoretical and practical consideration of anthelmintics. *De Lamar Lectures*, 1927–28. Baltimore.

LAMSON, P. D., and BROWN, H. W., 1936, Methods of testing the anthelmintic properties of ascaricides. *Amer. J. Hyg.*, **XXIII.**, 85.

LAMSON, P. D., and WARD, C. B., 1932, The chemotherapy of helminth infestations. *J. Parasit.*, **XVIII.**, 173.

LAMSON, P. D., and WARD, C. B., 1936, Earth worms as test objects for determining the value of drugs to be used in human intestinal helminth infestations. *Science*, **LXXXIV.**, 293.

LANE, C., 1932, Hookworn infection. London.

LANE C., 1935, The appraisement of hookworm killing drugs. *Lancet*, *i.*, 1459.

MAPLESTONE, P. A., and MUKERJI, A. K., 1933, Carbon tetrachlorethylene in the treatment of hookworm infection. *Indian med. Gaz.*, **LXVIII.**, 617.

PERRONCITO, E., 1880, Nota sull'azione dell'estratto etereo di felce maschio nei malatti di oligoemia epidemica provenienti dal Gottardo. *Osservatore*, Turin, **XVI.**, 801.

ROBERTS, F. H. S., 1932, Experiments in the treatment of stomach worms in sheep. *Qd Dept. Agric. Stock ; Div. Ent. Plant. Path.*, Pamphlet No. 5, p. 17.

SCHÜFFNER, W., and VERWOORT, H., 1913, Das Oleum chenopodii gegen Ankylostomiasis und eine neue Methode der Wertbestimmung von Wurmmitteln. *Münch. med. Wschr.*, **LX.**, 129.

SEDDON, H. R., and ROSS, I. C., 1928, Observations on the treatment of parasitic gastro-enteritis in sheep. *Vet. Res. Rep. N.S.W.*, No. 5, 62.

STOLL, N. R., and HAUSHEER, W. C., 1926, Accuracy in the dilution egg counting method. *Amer. J. Hyg.*, **VI.**, March supp., 80.

THE TREATMENT OF ASCARIASIS

Infection with *Ascaris lumbricoides* is extremely common throughout the world except in regions with cold climates. In parts of India and China it has been estimated that as much as 90 per cent. of the population is infected and, though infection does not necessarily cause any discomfort to the host, complications of very many kinds are attributed to the presence of the worms. The chief of these is mechanical disturbance caused by migration of the worms, which may penetrate into many parts of the body, causing either obstruction or, in some cases, bacterial infection.

In the treatment of hookworms with carbon tetrachloride, Lamson, Minot and Robbins (1928) pointed out that undesirable migrations of *Ascaris lumbricoides,* with obstruction of the gut or air passages, are by no means uncommon. Certain of the fatalities following administration of carbon tetrachloride have in fact been attributed to the presence of large numbers of ascaris, and there is now general agreement, among those carrying out hookworm campaigns, that either ascaris should be removed before the hookworms are treated, or else a drug should be used that will act simultaneously on both hookworms and ascaris.

Up to the present only three drugs have been extensively used in the treatment of ascaris infections, oil of chenopodium, santonin and hexylresorcinol, and none of these drugs is entirely free from all danger.

OIL OF CHENOPODIUM

Oil of chenopodium is obtained principally from *Chenopodium ambrosioides*, var. *anthelminticum* or American wormseed, commonly known as " Jerusalem oak " or " Mexican tea." *

Although there are still some dissentients (*cf.* Lüdtke, 1933) who believe that an ascaridole-free fraction of the oil possesses anthelmintic properties equal to those of the crude oil, the general consensus of opinion is in favour of the view that anthelmintic action is dependent on the ascaridole content.

---

* *Chenopodium album* is known to have been cultivated in Denmark from c. 1500 B.C. onwards.

Unfortunately, the ascaridole content of oil of chenopodium varies within very wide limits. Chopra and Mukerji (1932), for instance, found that Indian chenopodium is often very inferior to the American oil. The ascaridole content of a mixed Indian oil was only 46 per cent. as against 65 per cent. from American oil. A variety of *C. ambrosioides* is cultivated in Java, however, and is said to yield a good quality oil.

Since an effective method of estimating ascaridole is available (*cf.* Paget, 1926) and ascaridole itself is a commercial article there is now no excuse for using an oil of inferior quality.

Weiland, Broughton and Metzger (1935) found that the yield of oil is increased if potassium salts are added to the soil, which must also have a nitrogen phosphorus ratio of 1 : 4. The oil is found in greatest amount in the seeds, leaves and stems, in that order.

Although von Unruh (1932), who, in Mexico, used ascaridole itself in doses of 0·6 gm., found it a safe, uniform and reliable remedy for ascaris, there have been a number of toxic reactions, many of them, such as acute mania and deafness, being referable to the central nervous system (Bergmann, 1933). Hillenbrand (1932) regards the action as cumulative. Since hydrogen peroxide is very toxic to *Ascaris lumbricoides in vitro* and ascaridole itself is a peroxide, it has been suggested that the action of ascaridole may be due to its liberation of hydrogen peroxide. Butz and La Lande (1934), however, found that ascaridole has a greater toxicity than would be the case if its action were solely due to the generation of hydrogen peroxide. Substances of pronounced toxicity to *Ascaris lumbricoides* are nevertheless formed when oxygen or air is passed over α-pinene, turpentine oil or *d*-limonene, though the toxicity is not regarded as due to the action of peroxides.

Oil of chenopodium is probably superior to santonin in removing ascaris from man and from all animals on which it has been tested. For the ascaris infections of dogs and cats it is as effective as carbon tetrachloride, but not as effective as either carbon tetrachloride or carbon bisulphide in removing ascaris from horses.

According to Manson (1934) oil of chenopodium in doses of

3 c.c. is more effective against ascaris in man than tetrachloroethylene in doses of 4 c.c.

Wood (1931) finds that in horses oil of chenopodium acts efficiently on *Strongyles trichonemas* and other allied species : in pregnant mares it is very liable to produce toxic symptoms.

While oil of chenopodium, provided its ascaridole content is sufficient, is as effective as carbon tetrachloride against the Old World hookworm, *Ancylostoma duodenale*, it appears to be less effective against the New World species, *Necator americanus*.

Kubota (1935) claims that the essential oil obtained from *Xanthoxylum schiniifolia*, which in the Far East has long been endowed with anthelmintic properties, is as efficient as chenopodium oil.

References

Bergmann, O., 1933, Oleum Chenopodii als Ursache einer Gehörstörung. *Münch. med. Wschr.*, **LXXX.**, 734.

Butz, L. W., and La Lande, Jr., W. A., 1934, Anthelmintics I. The effect of hydrogen peroxide and some oxygenated terpene hydrocarbons upon *Ascaris lumbricoides*. *J. Amer. pharm. Ass.*, **XXIII.**, 1088.

Chopra, R. N., and Mukerji, B., 1932, Indian chenopodium. *Indian med. Gaz.*, **LXVII.**, 5.

Hillenbrand, K., 1932, Schwerhörigkeit nach Chenopodiumölvergiftung. *Münch. med. Wschr.*, **LXXIX.**, 1152.

Kubota, S., 1935, "Han Yao" the Chinese Materia Medica from which modern medicines have been prepared in Nippon in recent years. *Trans. 9th Congr. Far East. Ass. trop. Med.*, **II.**, 639.

Lamson, P. D., Minot, A. S., and Robbins, B. H., 1928, Prevention and treatment of carbon tetrachloride intoxication. *J. Amer. med. Ass.*, **XC.**, 345.

Lüdtke, H., 1933, Vergleichende Untersuchungen ueber das Ol. Chenopodii und sein wirksames Prinzip, das Askaridol. *Med. Klin.*, **XXIX.**, 611.

Manson, D., 1934, A comparative record of anthelmintic treatment with tetrachlorethylene and oil of chenopodium. *Indian med. Gaz.*, **LXIX.**, 500.

Paget, H., 1926, The determination of ascaridole in chenopodium oil. *Analyst*, **II.**, 170.

Unruh, V. von., 1932, Askariasis in subtropischen Mexiko. *Arch. Schiffs- u. Tropenhyg.*, **XXXVI.**, 4.

Weiland, G. S., Broughton, L. B., and Metzger, J. E., 1935, Wormseed oil production. *Indiana Agric. Exp. Sta. Bull.*, **CCCLXXXIV.**, 315.

Wood, W. A., 1931, Some notes on the treatment of equine strongylosis with oil of chenopodium. *2nd Rep. Dir. Inst. Anim. Path. Cambridge*, p. 113.

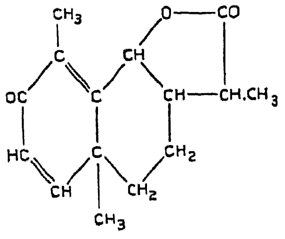
SANTONIN

Although santonin was only isolated by Kehler and Alms in 1829, the anthelmintic value of the plant from which santonin is obtained, *Artemisia maritima*, var. *anthelminticum* Linn., was well known to the Greeks and Romans, from whom a knowledge of its properties was derived by the Arabian physicians of the Middle Ages.

Recent observations on the anthelmintic properties of santonin have in part confirmed the reputation which it has so long enjoyed, for Caius and Mhaskar (1923) cured 16 out of 20 cases infected with ascaris. Brown (1934), however, who would place the cure rate at between 60 and 80 per cent., regards santonin as inferior both to hexylresorcinol and chenopodium in the treatment of ascaris infections: in animals results have not been very satisfactory. Many of the worms expelled are injured, but not necessarily killed, while *in vitro* santonin seems to have very little direct action on ascaris. In addition, santonin not infrequently produces disordered vision, xanthopsia. As a result numerous attempts have been made to prepare other substances with the same chemical groupings as santonin, but devoid of its unpleasant action and possessed of a higher anthelmintic capacity.

These attempts have met with considerable difficulties because agreement has been lacking as to the exact chemical constitution of santonin. The formula now generally adopted is that put forward by Clemo, Haworth and Walton (1929 and 1930), and confirmed by Wedekind and Tettweiler (1931).

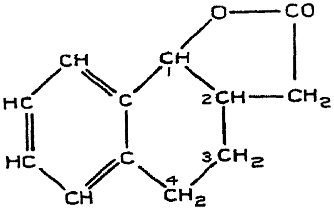
Formula I



Among the various efforts to produce lactones endowed with anthelmintic activities are those of von Oettingen and Garcia (1929), who studied the action of β-angelicalactone and the dilactone of acetonediacetic acid in ascaris infections of cats. The dilactone was the more efficient compound, and in seven out of ten experiments in cats all round worms were removed, a single dose of 0·3 gm. per kilogram of body-weight being sometimes sufficient. β-angelicalactone was irritating to the gastric mucosa and the M.L.D. in the cat was only 0·7–1·25 gm. per kilogram of body-weight; that of the dilactone was considerably greater, for 2·6 gm. per kilogram of body-weight produced only a transient slight depression.

Gluschke (1932) prepared a considerable number of lactone derivatives, of which two, both derived from α-tetralone, were as active anthelmintically as santonin. These compounds were "syntonin-a" (1 : 2 : 3 : 4-tetrahydro-1-hydroxynaphthalene-2-acetic acid lactone) and "syntonin-b" (1 : 2 : 3 : 4-tetrahydro-1-hydroxynaphthalene-2-methyl acetic acid lactone). The use of α-lactones of decahydro-1-hydroxy-2-naphthalene acetic acids of the formula $[C_{10}H_{16}.O.CO.CH]R$, where R may be either H, or an alkyl radical, has also been suggested [British Patent 378,088/1932]. In addition, Rosenmund and Schapiro (1934) have combined certain phenols and phenolic ethers with lactones derived from butyric acid. A striking difference was noted in the anthelmintic properties of the lactones combined with *ortho-*, *meta-* and *para-*cresols. *o*-Cresyl butyrolactone was considerably more active than santonin, the *para-*compound less efficient and the *meta-*compound practically inactive.

Syntonin-a



Clinical tests of the anthelmintic activities of these various synthetic lactones have not yet been carried out.

No satisfactory explanation of the anthelmintic action of santonin has yet been given. Rosenmund (1935) has, however, suggested that the electrical characters of the phenol lactones are of great importance in relation to their anthelmintic action. With this object he has studied the dipole moments of these compounds and claims that measurements of these moments allow the physico-chemical properties to be predicted.

References

BROWN, H. W., 1934, Intestinal parasitic worms in the United States; their diagnosis and treatment. *J. Amer. med. Ass.*, CIII., 651.

CAIUS, J. F., and MHASKAR, K. S., 1923, Notes on ascaricides. *Indian J. med. Res.*, XI., 377.

CLEMO, G. R., HAWORTH, R. D., and WALTON, E., 1929, The constitution of santonin. I. The synthesis of *dl*-santonous acid. *J. chem. Soc.*, p. 2368.

CLEMO, G. R., HAWORTH, R. D., and WALTON, E., 1930, The constitution of santonin. II. The synthesis of racemic *desmotropo*santonin. *Ibid.*, p. 1110.

GLUSCHKE, A., 1932, Die synthetische Darstellung neuer Wurmmittel und deren pharmakologische Auswertung. Ein Beitrag zur Kenntnis der Beziehungen zwischen chemischer Konstitution und vermifuger Wirkung in der Santoningruppe. II. Mitteilung. *Arch. wiss. prakt. Tierheilk.*, LXV., 201.

OETTINGEN, W. F. VON, and GARCIA, F., 1929, The toxicity and vermicidal properties of the dilactones of acetone, diacetic acid and β-angelicalactone in cats. *J. Pharmacol.*, XXXVI., 355.

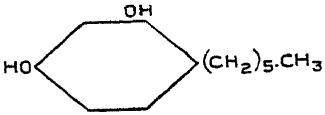
ROSENMUND, K. W., 1935, Studien über den Zusammenhang von dielectrischer Polarisation und pharmakologischer Wirkung. *Angew. Chem.*, XLVIII., 701.

ROSENMUND, K. W., and SCHAPIRO, D., 1934, Synthetische Versuche in der Reihe der Antihelmintika. *Arch. Pharm., Berl.*, CCLXXII., 313.

WEDEKIND, E., and TETTWEILER, K., 1931, Konstitution und oxydativer Abbau des Santonins. *Ber. dtsch. chem. Ges.*, LXIV., 1796.

HEXYLRESORCINOL

In 1921, Johnson and Lane studied the effects of introducing ethyl, propyl and butyl radicals into the resorcinol molecule in the *para*-position. Bactericidal action increased with increase in length of the alkyl chain. Leonard (1924) subsequently showed that, with the introduction of the hexyl radical into the resorcinol molecule, a peak of germicidal activity was reached, after which there was a rapid falling off.



Hexylresorcinol, 2 : 4-dihydroxy-*n*-hexylbenzene, was first employed as a urinary antiseptic and has since been extensively employed in the treatment of *Bacterium coli* infections of the urinary tract.

Although its use as an anthelmintic was suggested by Hall and Wigdor (1920), its action on worms was not tested till some years later, when Lamson, Ward and Brown (1930) found that it removed *Ascaris lumbricoides.* Since then it has been used as an anthelmintic in many parts of the world and has been found to reduce the egg counts by from 81 to 100 per cent., while from 53 to 100 per cent. of persons, it is claimed, have been completely cured.

THE EFFECT OF HEXYLRESORCINOL ON *ASCARIS LUMBRICOIDES*

No. of cases treated	Region	Method of testing results	Adult dose in gram.	Egg-count reduction	Negative	Author
82	U.S.A. Alabama	Stoll	1·0	I* 96	70	Lamson, Caldwell, Brown & Ward (1931).
1,350	,, Kentucky	,,	1·0	I 90	65	Lamson, Brown, Robbins & Ward (1931).
650	,, Louisiana	,,	1·0	I 90	69	Brown (1932).
1,784	Central America	,,	1·0	I 96	72	Molloy (1933).
160	,, ,,	,,	1·0	I 90	70·6	
31	Egypt	,,	1·4	I 94·5	69	Azim (1933).
				II 99·3	90	
				III 100	100	
21	India	,,	1·0	I 94	66·6	Maplestone & Mukerji (1932).
62	Ceylon	,,	0·6–0·9 (children)	I 81	79	Lamson, Brown & Ward (1935).
30	Siam	,,	1·0	I 89	71	Lamson, Brown & Ward (1935).
100	Samoa	,,	1·0	I 86		
				II 99		
4	Fiji	Worm count	2·0	I 100	100	Lamson, Brown & Ward (1935).
74	China	—	1·0	I —	78	Wang (1933).
861	Philippines	—	1·0	I 82	53 64	Tubangui, Basaca & Pasco (1934).

* The numeral indicates number of treatments.

It is perhaps unfortunate that the majority of investigators who have studied the effects of hexylresorcinol in man have been content to employ only the Stoll method of egg counting, the efficiency of which is by no means universally accepted (Maplestone, 1932). The ideal to be aimed at is undoubtedly the removal of every worm.

An exact determination of this point entails, however, prolonged observation, for as Brown (1934) has pointed out, worms may be evacuated for as long as ten days after treatment. Nevertheless, such observations are urgently required if a correct estimation of the efficacy of hexylresorcinol is to be obtained.

Lamson and Brown (1936) point out that when the dose of hexylresorcinol is proportional to body-weight the drug is more efficient in adults than in children. Maplestone and Mukerji (1931) obtained similar results. The most probable explanation is that a child or young animal has a relatively longer gut in proportion to body-weight than an adult.

Apart from its irritant local action, the main drawback to the extended use of hexylresorcinol lies in the fact that starvation for at least twelve hours before treatment is essential if food is not to interfere with anthelmintic action.

Lamson and Ward (1932) believe that hexylresorcinol acts primarily on the cuticle of *Ascaris lumbricoides*, raising blisters. *In vitro* when immersed in dilute solutions of the drug the worms become stiff and immovable : they do not recover when removed. Hexylresorcinol may therefore be regarded as a true vermicide (*cf.* Faust, 1931).

Action on other Parasitic Helminths. Unlike many other anthelmintics, the action of hexylresorcinol is not confined to *Ascaris lumbricoides*, for a number of other parasitic helminths are affected in varying degree. With a 1 gm. dose of hexylresorcinol, Lamson, Caldwell, Brown and Ward (1932) obtained egg-count reductions in *Necator americanus* infestations of from 16·6 to 97·7 per cent. With 0·8 gm. doses the reduction was from 26·5 to 90·1 per cent. Molloy (1933) compared fæcal egg counts in 1,455 persons before and after one and two treatments with hexylresorcinol. One treatment so lessened the hookworm-load that it relieved most symptoms but did not eliminate all infective risk : of 618 persons who had had two treatments 500 still remained infected. Brown (1931) showed that hookworm eggs and larvæ (*Necator*) as well as Ascaris eggs are rapidly killed by dilute solutions of hexylresorcinol.

Ancylostoma duodenale is not as effectively treated as *N. americanus* (Maplestone and Mukerji, 1929, Biggam and Ghalioungui, 1932, Azim, 1933). Yutuc and Tubangui (1935), however, treated dogs infected with *Ancylostoma caninum* and *A. braziliensis* with hexylresorcinol and found at post-mortem examination that in 10·5 per cent. all worms had been removed.

With *Trichuris* a single dose of hexylresorcinol has shown an egg-count reduction as high as 30 to 50 per cent. Molloy (1933) regards hexylresorcinol as, with the exception of the latex of the wild fig, the most effective drug against whip-worms. Brown (1932) has also shown that hexylresorcinol is very effective in *Enterobius* infections. The drug is given by enema in a dilution of 1 : 1,000 and by mouth in doses of 0·1 gm. per year of age, with a maximum dose of 1 gm.

Against tapeworms, such as *Tænia saginata,* hexylresorcinol is not as effective as carbon tetrachloride (Wright, Bozicevich and Underwood, 1931, Tubangui, Basaca and Pasco, 1934).

Hexylresorcinol is highly toxic for cats, but for rats, dogs and men its toxicity is very slight. In doses of 1 gm. per kilogram of body-weight, Maplestone and Chopra (1934) found that cats show no erosion of the gastric mucosa, but local action depends on local concentration, and when a large dose is introduced directly into the cat's stomach necrosis of the mucosa follows. Doses of 0·325 to 0·520 gm. per kilogram were fatal to cats, a result confirmed by Lamson, Brown and Ward (1935). These workers have, however, given as much as 5 gm. to dogs weighing 10 kgm. without ill-effects. One dog was given 1 gm. daily for two hundred days : it gained in weight and showed no symptoms, while at autopsy there were no lesions. These findings differ from those obtained by Christensen and Lynch (1933), who killed a dog with five bi-weekly doses of 0·8 gm. and reported severe pathological lesions of the heart and liver in others. Wright, Bozicevich and Underwood (1931) found that a pig weighing 21·8 kgm. tolerated a single dose of 13 gm. The slight changes found in the heart and liver after repeated oral administration were not regarded as due to the action of hexylresorcinol.

Clapham (1932), in mice killed with lethal doses, noted petechial hæmorrhages in the stomach, fatty degeneration and necrotic foci in the convoluted tubules of the kidney, cloudy swelling and fatty degeneration in the liver.

Lamson, Brown and Ward (1935) conclude that though superficial irritation of the gastro-intestinal tract with small submucous hæmorrhages may occur after oral ingestion of therapeutic doses of hexylresorcinol, no permanent pathological lesions result. Urine from both dogs and men, after oral doses of hexylresorcinol, shows no albumen, while renal function studies have failed to reveal any abnormality.

Although animal experiments are of value in determining the relative toxicity of drugs and in showing the types of lesion produced, they do not necessarily give as good an idea of their safety in human medicine as actual administration to man. Leonard (1924) reported the daily administration to men of 0·15 to 1 gm. of hexylresorcinol three times daily over a period of ten weeks, till 70 gm. had been taken, without ill effect. There was no cumulative action. Ashford, Payne and Payne (1933) even gave hexylresorcinol to patients who were so anæmic from hookworm disease as to require transfusion. Lamson, Brown and Ward (1935) state that over 10,000 patients have now been treated with crystalline hexylresorcinol for intestinal helminths without evidence of toxic symptoms.

When taken as crystals, either in gelatin capsules or in the form of pills, hexylresorcinol causes no sensation in the stomach of most individuals. If, however, the pills are chewed there may be whitening and superficial erosion of the oral mucosa, while occasionally burning of the stomach is felt, and in a few cases vomiting may occur, especially in those who have eaten food shortly before taking the drug. These effects are due to the fact that hexylresorcinol readily precipitates protein. Diarrhœa may also occasionally follow a single administration. The ingestion of alcohol does not increase the toxicity of hexylresorcinol.

After oral administration, both in man and dogs, from 25 to 30 per cent. of the drug is absorbed from the intestinal tract and excreted in the urine as the ethereal ester ; the other 70 to 75 per cent. is excreted unchanged in the stools.

The chief drawback to the administration of the drug is, apart from its irritant action on mucous membranes, its very objectionable taste. It is fairly insoluble in water (1 in 2,000), and though freely soluble in olive oil it cannot be given in this menstruum, for it remains in the oil and fails to attack the worms. The best method of administration appears to be in the form of a pill made up of the crystals and covered with a thin sugar coating : such pills must not be chewed. An alternative method is to enclose the crystals in gelatin capsules, but there is then some reduction in anthelmintic efficiency. In hot, moist climates anthelmintic power is often rapidly lost.

OTHER ALKYLHYDROXYBENZENES

In an attempt to obtain compounds equally toxic to *Ascaris lumbricoides*, but without its local irritant action, Lamson and his colleagues (1935 and 1936) examined the ascaricidal action *in vitro* of a large number of hydroxybenzenes, including alkylresorcinols, alkylcresols, alkylphenols, polyalkylphenols, phenols with other than normal alkyl side-chains, alkylpolycyclicphenols, halogenated phenols, phenolic ketones, phenolic ethers and esters, and a number of organic acids.

Heptylresorcinol, though almost as irritating to mucous membranes as hexylresorcinol, was found by Lamson, Caldwell, Brown and Ward (1932) to be less effective in man, both against *Ascaris lumbricoides* and *Necator americanus*. In the case of heptylresorcinol, however, there is little, if any, absorption from the intestine and none can be detected in the urine. Heptylresorcinol dissolves as readily in fats as hexylresorcinol and then loses its anthelmintic action entirely. David and Johnstone (1933) found heptylresorcinol quite ineffective in trichuriasis.

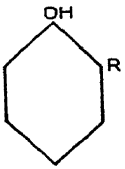
Among a group of miscellaneous polyhydroxybenzenes, 4-*n*-

hexylcatechol was the only one that had any marked ascaricidal action *in vitro :* it was too irritant for use in man.

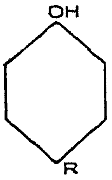
In the *ortho-* and *para-n-*alkylphenol series ascaricidal properties were found to increase with the introduction of normal alkyl radicals into both the *ortho-* and *para-*positions of phenol up to and including *n-*amylphenol. In the higher members of the series ascaricidal action rapidly diminished. Coulthard, Marshall and Pyman (1930), it is of interest to note, similarly found an increase in bactericidal activity and a decrease in toxicity with increase in length of the side-chains in the *n-*alkylphenol series.

Although the *in vitro* ascaricidal properties of the *ortho-* and *para-*alkylphenols are very nearly the same, the *para-*compounds appear slightly the stronger. On the other hand, the *ortho-*series are less irritating locally than the *para-*series, local irritant action decreasing with increase in length of the alkyl radicals. *ortho-n-*Heptylphenol is the lowest member of the series that causes no whitening of the mucous membranes of the mouth, nor are gross or microscopic lesions found in the gastric mucosa of dogs after oral administration of 3·0 c.c.

For white rats the toxicity of these phenols, given by mouth, decreases with increase in length of the alkyl chain.



*ortho-n-*alkylphenols.

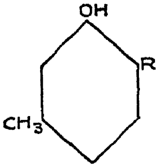


*para-n-*alkylphenols.

In man 90 per cent. of *ortho-n-*heptylphenol was found to be absorbed from the intestinal tract after oral administration, but only 10 per cent. was recovered in the urine and 12 per cent. in the fæces.

The anthelmintic action of *ortho-n-*heptylphenol in man has been found by Lamson, Molloy and Brown (1935) to be considerably less than that of hexylresorcinol, for even when 4·2 c.c. were given, as compared with 1 gm. of hexylresorcinol, only 38 per cent. of *Ascaris lumbricoides* were removed in contrast with 90 per cent. removed by 1 gm. of hexylresorcinol, 58 per cent. of *Necator americanus*, as against 70 to 80 per cent. by hexylresorcinol and 32 per cent. of *Trichuris trichiura*, compared with 30 to 50 per cent. removed by hexylresorcinol.

In the 6-*n*-alkyl-*m*-cresols also, Lamson and Brown (1935) found an increasing ascaricidal action to be correlated with increase in length of the alkyl radical from *m*-cresol up to butyl-*m*-cresol, after which there is a falling off in activity. A similar increase in activity on *Ascaris lumbricoides in vitro* was shown by Lamson and Brown (1936). The toxicity of the series decreases with increase of the alkyl radical, as does the local irritant action, 6-*n*-hexyl-*m*-cresol being the lowest member of the series which does not cause whitening of the oral mucosa. The lower toxicity of the higher cresols is not entirely due to decreased water solubility, or to decreased absorption, for Robbins (1934) found that after 2 gm. doses, orally administered, man absorbs about 90 per cent. of 6-*n*-hexyl-*m*-cresol. Broom (1931) found that rats given 0·5 gm. of amyl-*m*-cresol per kilogram of body-weight by stomach tube survived for at least thirty-seven days : the same dose of hexylresorcinol was toxic. Amyl-*meta*-cresol had no cumulative action and was rapidly absorbed and eliminated. From a comparative study of the toxicity of 6-*n*-hexyl-*m*-cresol and hexylresorcinol in animals and man, Brown and Lamson (1935) conclude that 6-*n*-hexyl-*m*-cresol is no more toxic than hexylresorcinol. It has in fact been given to 100 persons in doses up to 4·2 c.c. without producing symptoms or any complaint. Unfortunately, in man 6-*n*-hexyl-*m*-cresol is a less efficient ascaricide than hexylresorcinol, and in doses of 4 c.c. it removed only 55 per cent. of *Ascaris*, 70



6-*n*-alkyl-*m*-cresols.

per cent. of *Necator* and 30 per cent. of *Trichuris* (Lamson, Molloy and Brown, 1935). As in the case of resorcinol 6-*n*-hexyl-*m*-cresol, when given in proportion to body-weight, is more effective in larger than in smaller animals (Lamson and Brown, 1936). Unlike hexylresorcinol, 6-*n*-hexyl-*m*-cresol and also *ortho*-heptylphenol have a greater action on *Necator americanus* than on *Ascaris lumbricoides*: they thus resemble thymol. Among polyalkylphenols, Lamson, Brown and Ward (1935) found some which showed a considerable degree of activity against pig ascaris *in vitro*, but no single substance combined in itself high ascaricidal activity, low toxicity and inapparent local irritant action to such a degree as to indicate that it would be a more practical anthelmintic than hexylresorcinol in treating human ascariasis. Among phenols other than those containing normal alkyl side-chains there were again certain compounds, such as *p-tert*-amylphenol, that showed ascaricidal properties, but none were more satisfactory than hexylresorcinol. The introduction of alkyl radicals into certain polycyclic phenols led to no increase in ascaricidal action : the introduction of halogens into certain alkylphenols was, however, more promising, and ascaricidal action *in vitro* was particularly shown by *p*-chlorocarvacrol, 4-chloro-2-hexylphenol and 4-chloro-2-heptylphenol.

The ascaricidal action of 5-chlorocarvacrol has also been studied by Kochmann (1931), who finds that *in vitro* ascaris is paralysed in concentrations of 1 in 150,000, while higher concentrations are rapidly lethal. In dogs non-toxic doses possessed a definite ascaricidal action. Chlorocarvacrol was found to be less irritant than either carvacrol itself or thymol.

Certain other alkylpolyhydroxyhalogenobenzenes such as 1 - octyl - 5 - chlororesorcinol, or 5 - octyl - 4 - chloropyrocatechol (British Patent 415715 : 1934) may also have an anthelmintic action.

Ascaricidal action was not shown by more than 100 phenolic ketones, ethers and esters, though some thirty organic acids and twenty esters of simple aromatic and aliphatic acids had a very slight ascaricidal activity. Two monoethers of dihydric phenols, namely, quinol monoamyl ether and hexylresorcinol monoethyl ether, were, however, highly ascaricidal *in vitro*, but, unfortunately, were too toxic for use *in vivo*.

Though this exhaustive search has not yet ended in the finding of a hydroxybenzene derivative more satisfactory than hexylresorcinol in the treatment of human ascariasis, certain conclusions may be drawn from these studies.

In the first place, it seems that a certain degree of local irritant action is inseparable from anthelmintic activity.

With increase of the alkyl side-chain there is increase in ascaricidal activity to a maximum, after which there is a rapid loss of activity. Thus in the series of 4-*n*-alkylresorcinols, resorcinol itself and propylresorcinol are inactive against pig ascaris *in vitro*, butylresorcinol possesses a slight activity while amyl-, hexyl- and heptylresorcinol are all markedly ascaricidal. With octylresorcinol the activity decreases and is absent in duodecylresorcinol and the higher members of the series.

Substances with good ascaricidal activity may have a wide range of solubility in water varying from 1 : 1,000 to 1 : 35,000.

Melting point is probably of greater importance than solubility for, as was first pointed out by Harwood (1934), all those substances which show activity have relatively low melting points, usually below 80° C.

The lower members of the series have, as a rule, a more intense irritant local action than the higher members, and, according to Brown and Lamson (1935), the lower members of the series are more toxic than the higher to rats, although Anderson, David and Leake (1931) found in guinea-pigs that as far as hexyl-, heptyl- and octylresorcinol were concerned the toxicity by mouth increased.

This variation in toxicity with difference in the length of the alkyl radical and difference of animal species is of considerable interest, since it shows that the " law " enunciated by Richardson (1869), to the effect that increase in toxicity is associated with increase in the length of the alkyl radical of normal alcohols as well as of other series, may be correct for one series of compounds when tested in a given manner in a certain species of animals. There is, however, no justification for assuming that such a " law " will apply to the reactions of other homologous series in the same or different species of animals, especially when the drugs are given by different routes.

References

Anderson, H. H., David, N. A., and Leake, C. D., 1931, Oral toxicity of certain alkyl resorcinols in guinea-pigs and rabbits. *Proc. Soc. exp. Biol., N.Y.*, **XXVIII.**, 609.

Ashford, B. K., Payne, G. C., and Payne, F., 1933, The larval phase of uncinariasis. *Puerto Rico J. Publ. Hlth.*, **IX.**, 97.

Azim, M. A., 1933, Investigations on the anthelmintic power of hexylresorcinol. *J. Egypt. med. Ass.*, **XV.**, 635.

Biggam, A. G., and Ghalioungui, P., 1932, Hexylresorcinol in the treatment of *Ancylostoma duodenale* infection. *J. trop. Med.*, **XXXV.**, 353.

Broom, W. A., 1931, A note on the toxicity of amyl-*meta*-cresol. *Brit. J. exp. Path.*, **XII.**, 327.

Brown, H. W., 1931, Effect of hexylresorcinol upon ascaris and hookworm eggs. *Proc. Soc. exp. Biol., N.Y.*, **XXVIII.**, 1036.

Brown, H. W., 1932, Treatment of pinworm (*Enterobius vermicularis*) infestation with hexylresorcinol. *Ibid.*, **XXX.**, 221.

Brown, H. W., 1932, The treatment of ascariasis and trichuriasis with hexylresorcinol pills. *Amer. J. Hyg.*, **XVI.**, 602.

Brown, H. W., 1934, Intestinal worms in the United States; their diagnosis and treatment. *J. Amer. med. Ass.*, **CIII.**, 651.

Brown, H. W., and Lamson, P. D., 1935, The oral toxicity of 6-alkyl-*meta*-cresols. *J. Pharmacol.*, **LIII.**, 265.

Christensen, B. V., and Lynch, H. J., 1933, The effect of anthelmintics on the host. I. Tetrachlorethylene: II. Hexylresorcinol. *Ibid.*, **XLVIII.**, 311.

Clapham, P. A., 1932, Hexylresorcinol as a general vermicide. *J. Helminth.*, **X.**, 195.

Coulthard, C. E., Marshall, J., and Pyman, F. L., 1930, The variation of phenol coefficients in homologous series of phenols. *J. chem. Soc.*, p. 280.

David, N. A., and Johnstone, H. G., 1933, Heptylresorcinol in the treatment of hookworm infection. *Amer. J. Hyg.*, **XVII.**, 287.

Faust, E. C., 1931, *In vitro* effects of certain drugs on strongyloides. *Proc. Soc. exp. Biol., N.Y.*, **XXVIII.**, 691.

Hall, M. C., and Wigdor, M., 1920, Studies on anthelmintics. VII. A comparison of castor oil and other purgatives in connection with the administration of some anthelmintics. *J. Amer. vet. med. Ass.*, **V.**, 110.

Harwood, P. D., 1934, Effect of certain physical factors on the *in vitro* testing of anthelmintics. *Proc. Soc. exp. Biol., N.Y.*, **XXXII.**, 131.

Johnson, T. B., and Lane, F. W., 1921, The preparation of some alkyl derivatives of resorcinol and the relation of their structure to antiseptic properties. *J. Amer. chem. Soc.*, **XLIII.**, 348.

Kochman, M., 1931, Chlorcarvacrol als Anthelminticum (Carvasept) (nebst Bemerkungen über die Wirkung des Carvacrol, Thymol und Santonin). *Arch. exp. Path. Pharmak.*, **CLXI.** 196.

LAMSON, P. D., and BROWN, H. W., 1935, Anthelmintic studies on alkylhydroxybenzenes. III. 6-*n*-alkyl-*meta*-cresols. *J. Pharmacol.*, LIII., 227.

LAMSON, P. D., and BROWN, H. W., 1936, Methods of testing the anthelmintic properties of ascaricides. *Amer. J. Hyg.*, XXIII., 85.

LAMSON, P. D., BROWN, H. W., ROBBINS, B. H., and WARD, C. B., 1931, Field treatments of ascariasis, ancylostomiasis and trichuriasis with hexylresorcinol. *Ibid.*, XIII., 803.

LAMSON, P. D., BROWN, H. W., STOUGHTON, R. W., HARWOOD, P. D., BALTZLY, R., and BASS, R., 1935,[1] Anthelmintic studies on alkylhydroxybenzenes. II. *Ortho*- and *para*-*n*-alkylphenols. *J. Pharmacol.*, LIII., 218.

LAMSON, P. D., BROWN, H. W., STOUGHTON, R. W., HARWOOD, P. D., BALTZLY, R., and BASS, R., 1935,[2] Anthelmintic studies on alkylhydroxybenzenes. IV. Isomerism in polyalkylphenols. *Ibid.*, LIII., 234.

LAMSON, P. D., BROWN, H. W., STOUGHTON, R. W., HARWOOD, P. D., BALTZLY, R., and BASS, R., 1935,[3] Anthelmintic studies on alkylhydroxybenzenes. V. Phenols with other than normal alkyl side-chains. *Ibid.*, LIII., 239.

LAMSON, P. D., BROWN, H. W., and WARD, C. B., 1935, Anthelmintic studies on alkylhydroxybenzenes. I. Alkylpolyhydroxybenzenes. *Ibid.*, LIII., 198.

LAMSON, P. D., BROWN, H. W., WARD, C. B., and ROBBINS, B. H., 1930, Hexylresorcinol in the treatment of hookworm disease. *Proc. Soc. exp. Biol., N.Y.*, XXVIII., 191.

LAMSON, P. D., CALDWELL, E. L., BROWN, H. W., and WARD, C. B., 1932, A comparison of the anthelmintic properties of hexylresorcinol and heptylresorcinol. *Amer. J. Hyg.*, XV., 306.

LAMSON, P. D., MOLLOY, D. M., and BROWN, H. W., 1935, Field studies of the anthelmintic action of *ortho*-heptylphenol and 6-*n*-hexyl-*meta*-cresol against *Ascaris lumbricoides*, *Necator americanus* and *Trichuris trichiura*. *Ibid.*, XXI., 188.

LAMSON, P. D., and WARD, C. B., 1932, The chemotherapy of helminth infestations. *J. Parasit.*, XVIII., 173.

LAMSON, P. D., WARD, C. B., and BROWN, H. W., 1930, An effective ascaricide-Hexylresorcinol. *Proc. Soc. exp. Biol., N.Y.*, XXVII., 1017.

LEONARD, V., 1924, Hexylresorcinol. *J. Urol.*, XII., 585.

MAPLESTONE, P. A., 1932, The testing of anthelmintics. *Indian med. Gaz.*, LXVII., 673.

MAPLESTONE, P. A., and CHOPRA, R. N., 1934, The effect of hexylresorcinol in cats. *Indian J. med. Res.*, XXI., 519.

MAPLESTONE, P. A., and MUKERJI, A. K., 1929, Tetrachlorethylene in the treatment of hookworm disease. *Indian med. Gaz.*, LXIV., 424.

MAPLESTONE, P. A., and MUKERJI, A. K., 1931, The treatment of ascariasis. *Ibid.*, LXVI., 627.

MAPLESTONE, P. A., and MUKERJI, A. K., 1932, Hexylresorcinol as an anthelmintic. *Ibid.*, LXVII., 610.

MOLLOY, D. M., 1933, The treatment of hookworm and other intestinal infections with hexylresorcinol under field conditions in Central America. *Sth. med. J.*, XXVI., 575.

RICHARDSON, B. W., 1869, Lectures on experimental and practical medicine Physiological research on alcohols. *Med. Times and Gaz.*, II., 703.

ROBBINS, B. H., 1931, Quantitative studies on the absorption and excretion of hexylresorcinol and heptylresorcinol under different conditions. *J. Pharmacol.*, XLIII., 325.

ROBBINS, B. H., 1934, Quantitative studies on the absorption and excretion of certain resorcinols and cresols in dogs and man. *Ibid.*, LII., 54.

Tubangui, ., Basaca, M., and Pasco, A. M., 1934, Hexylresorcinol as an a ic. Its efficiency against the intestinal parasites of man. *pp. J. Sci.*, **LIV.**, 473.

K. C., 1933, The treatment of ascariasis with hexylresorcinol. *Chin. med. J.*, **XLVII.**, 1433.

ight, W. H., Bozicevich, J., and Underwood, P. C., 1931, Critical tests of miscellaneous drugs as anthelmintics for ascarids, whipworms and nodular worms of swine. *N. Amer. Vet.*, **XII.**, 27.

Yutuc, L. M., and Tubangui, M. A., 1935, The treatment of canine ancylostomiasis with hexylresorcinol. *Philipp. J. Anim. Industr.*, **II.**, 193.

ANKYLOSTOMIASIS

Hookworm disease, due either to *Necator americanus*, *Ancylostoma duodenale*, or more rarely to *A. braziliensis*, is one of the primary causes of the lowered vitality that is characteristic of the indigenous inhabitants of many tropical and sub-tropical areas in both the Old and New Worlds. The ideal anthelmintic is, of course, one which is capable either directly, or indirectly through its interaction with the tissues, of destroying all the infecting parasites without in any way injuring the host. The complete removal of all worms is of great importance for, while in a majority of cases the removal of a percentage of worms will relieve most symptoms, instances have been recorded where less than a dozen worms are known to have produced severe symptoms of anæmia.

While chloroform and such mixtures as Manson's chloroform and eucalyptol have been largely abandoned, oil of chenopodium is still used, despite the many toxic reactions recorded and a lack of critical evidence of its actual capacity for removing hookworms. β-Naphthol is now rarely administered : according to Milne (1933) the minimum lethal dose is 1·3 gm. and lower doses are inefficient. Thymol (3-methyl-6-*iso*propylphenol) was formerly widely used in the treatment of hookworm disease, and according to Lane (1935) it still remains the most satisfactory drug, owing not only to its efficiency but also to its low toxicity. Lane (1932), in fact, has been unable to find any constant symptomatology or post-mortem lesions associated with subjects who are said to have died as a result of the administration of thymol. He himself has given 60,000 administrations without any death. Ashford (1935) gave 1,600,000 doses in Puerto Rico and Howard (1919) 1,000,000 doses in the United States with similar results. Lane (1932) claims to have obtained complete removal of hookworms in about 50 per cent. of adults, while Caius and Mhaskar (1919, 1920 and 1923), using the Schüffner-Verwoort method of trial and 60 gr. of particulate thymol for the test treatment, obtained the following comparative results :—

Drug	Dose	Percentage of worms removed	
		Ancylostoma duodenale	*Necator americanus*
Thymol (particulate) .	4 gm.	96·1	99·6
Ascaridole . .	1 c.c.	60·9	83·4
Carbon tetrachloride .	1 c.c.	11·5	90·8
	3 c.c.	76·4	96·3
	4 c.c.	93·0	98·0
	5 c.c.	96·5	99·9

Lane believes that anthelmintic action is dependent on absorption, and that the actual vermicide is not thymol itself: finely powdered in doses of 60 gr., and mixed with an equal amount of sugar of milk, it is said to be readily absorbed. Direct evidence for these beliefs does not seem to be available. The claim of thymol to be an efficient anthelmintic for the treatment of hookworm does, however, require further critical investigation.

At present the drugs chiefly employed in the treatment of hookworm disease are the halogenated hydrocarbons, carbon tetrachloride, tetrachloroethylene, hexylresorcinol and oil of chenopodium: the two last are discussed in relation to the treatment of ascariasis. It should be remembered that in cases of hookworm disease removal of the parasites does not necessarily produce clinical improvement, nor does increased diet. The one necessity, as Rhoads, Castle, Payne and Lawson (1934) have pointed out, is administration of iron. If ferric ammonium citrate is given, with or without the removal of the parasites, the anaemia and the general clinical condition rapidly improve.

References

Ashford, B. K., 1935, quoted by Clayton Lane. *Lancet*, *i.*, 1459.

Caius, J. F., and Mhaskar, K. S., 1919, The correlation between the chemical composition of anthelmintics and their therapeutic values in connection with the hookworm inquiry in the Madras Presidency. I. Thymol. *Indian J. med. Res.*, VII., 429.

Caius, J. F., and Mhaskar, K. S., 1920, The correlation between the chemical composition of anthelmintics and their therapeutic values in connection with the hookworm inquiry in the Madras Presidency. II. Oleum Chenopodii. *Ibid.*, VII., 570.

Caius, J. F., and Mhaskar, K. S., 1923, The correlation between the chemical composition of anthelmintics and their therapeutic values in connection with the hookworm inquiry in the Madras Presidency. XX. Carbon tetrachloride. *Ibid.*, XI., 347.

Howard, H. H., 1919, The control of hookworm disease by the intensive method. Publication No. 8, Rockefeller Foundation, International Health Board.

Lane, C., 1932, "Hookworm infection," London.

Lane, C., 1935, The appraisement of hookworm killing drugs. *Lancet, i.*, 1459.

Milne, J. C., 1933, A case of beta-naphthol poisoning. *Malay. med. J.*, VIII., 73.

Rhoads, C. P., Castle, W. B., Payne, G. C., and Lawson, H. A., 1934, Observations on the etiology and treatment of anæmia associated with hookworm infection in Puerto Rico. *Amer. J. trop. Med.*, XIII., 317.

CARBON TETRACHLORIDE

Carbon tetrachloride, the first halogenated hydrocarbon to be extensively employed in combating hookworm disease, was introduced by Hall (1921) and has now been used in the treatment of many millions of human beings. Despite its wide use, the number of critical tests on its powers of removing hookworms has not been large. Caius and Mhaskar (1923) in India found that 4 c.c. followed by a purge expelled 93 per cent. of *Ancylostoma duodenale* and 98·1 per cent. of *Necator americanus* with a cure rate of 77·2 per cent. : after 5 c.c. and a purge the cure rate was 83·3 per cent.

Field (1924), with a dose of 4 c.c. and very careful after-examinations, obtained a cure rate of 77 per cent. in infections due to *N. americanus*. Maplestone and Mukerji (1929) gave 3 c.c. to sixty hospital patients, and using direct centrifugal floatation obtained a cure rate of 48 per cent. ; when 1 c.c. of oil of chenopodium of unstated ascaridole content was added, the cure rate among sixty patients was only 46·4 per cent. Although carbon tetrachloride is undoubtedly efficient, more especially against *N. americanus*, this efficiency is outweighed by its undoubted toxicity.

On the one hand, Lambert (1933) records more than 100,000 consecutive treatments with only six deaths that could be attributed to carbon tetrachloride. Tomb and Helmy (1933), however, report nineteen deaths in Egypt : on an average seven to eight deaths from carbon tetrachloride poisoning are reported every year in Egypt. Hussameddin (1931) had one death in 1,200 administrations, the dose being 2 c.c., Nag (1929) three deaths among 289 children. Lindsay (1935) from Paraguay reports that, among the underfed population, doses standardised according to age have been followed by many unexplained deaths in children and in adults. Carbon tetrachloride may also be a factor in explaining the large number of liver necroses found among the population of Brazil by Soper (1935) during the course of viscerotomy examinations for suspected cases of yellow fever. The only factor common to many of these cases was that the patients had been treated for hookworm disease. Findlay and Davey (1936) in Africa have also described a case of carbon tetrachloride poisoning suspected of being yellow fever.

It is now well recognised that carbon tetrachloride is capable of producing liver necrosis associated with a fall in the blood sugar and an increase in the guanidine content. This toxæmia can be combated by the administration of calcium salts—calcium gluconate especially appears to be of value—and is more likely to occur when the diet is deficient in calcium. Nevertheless, it is difficult to explain all the recorded deaths on the calcium deficiency theory. Tomb and Helmy (1933) divide the deaths into two types : (1) acute, occurring twenty minutes to six hours after administration ; (2) delayed, occurring two to three days after administration. The presence of a large amount of undigested fat in the stomach and intestine or heavy ascaris infection delaying elimination may possibly be predisposing causes. In dogs a meat diet increases the tendency to form guanidine, while a high carbohydrate diet decreases it. Hassan and Salah (1935) do not regard chemical impurities as of great significance : uncomplicated bilharzial cirrhosis is not a contra-indication, nor is there any close correlation between fatalities and the amount administered. According to Oxer (1933), deaths may occur in sheep without the occurrence of liver necrosis. Savignoni and Maria (1933) have brought forward evidence to show that carbon tetrachloride is especially toxic to the liver and kidneys of the fœtus and should not, therefore, be administered during pregnancy.

The most probable causes of liver necrosis following administration of carbon tetrachloride are (1) individual susceptibility and (2) liver insufficiency from intercurrent toxæmia.

Cameron and Karunaratne (1936) have shown that rats receiving repeated small doses of carbon tetrachloride differ considerably in the recuperative power of their livers, while if repeated insults are offered to the liver tissue in all animals there comes, sooner or later, a time when the liver cells become less and less capable of proliferating and repairing the damage. This crisis is likely to occur at an earlier period if carbon tetrachloride acts on a liver already damaged from some intercurrent toxæmia, and the number of toxic agents which can injure the liver is extremely large (*cf.* Findlay, 1933).

Klotz (1934) claims that when carbon tetrachloride is administered together with medicinal charcoal toxicity is removed, but anthelmintic efficiency is maintained: doses up to 5 c.c. per kilogram of body-weight produced no toxic symptoms in dogs, Forbes and Neale (1936), and Forbes, Neale and Scherer (1936) believe that they have isolated from hogs' liver a preparation which, in rats at any rate, protects the liver against the toxic action of carbon tetrachloride. The isolation of a crystallisable liver fraction is reported by Forbes and McConnell (1937). In view of the frequent fatalities that have been recorded, the use of carbon tetrachloride in the treatment of human beings is probably best restricted to specially selected cases. On the other hand, it must be remembered that the toxic reactions following carbon tetrachloride are relatively few in comparison with the large numbers of persons to whom it has been administered. This applies also to many other long established anthelmintics. Newer drugs, such as hexylresorcinol, may yet prove equally toxic if given to equal numbers of people.

Brown (1937) believes that carbon tetrachloride in oil, and probably also chenopodium, act on worms through the body wall rather than as a result of oral

References

Brown, H. W., 1937, Observations on the mode of action of several anthelmintics upon *Ascaris lumbricoides* (pig strain). *Amer. J. Hyg.*, XXVI., 72.

Caius, J. F., and Mhaskar, K., 1923, The correlation between the chemical composition of anthelmintics and their therapeutic values in connection with the hookworm inquiry in the Madras Presidency. XX. Carbon tetrachloride. *Indian J. med. Res.*, XI., 347.

Cameron, G. R., and Karunaratne, W. A. E., 1936, Carbon tetrachloride cirrhosis in relation to liver regeneration. *J. Path. Bact.*, XLII., 1.

Field, F. E., 1924, Carbon tetrachloride in the treatment of human ankylostome infection. *Brit. Guiana med. Annu.*, 1923, 174.

Findlay, G. M., 1933, Toxic jaundice. *Proc. R. Soc. Med.*, XXVI. (Sect. trop. Dis., 32).

Findlay, G. M., and Davey, T. H., 1936, Yellow fever in the Gambia. II. The 1934 outbreak. *Trans. R. Soc. trop. Med. Hyg.*, XXX., 151.

Forbes, J. C., and McConnell, J. S., 1937, Crystallisation of liver fraction protecting against necrosis from carbon tetrachloride on chloroform administration. *Proc. Soc. exp. Biol. Med.*, XXXVI., 359.

Forbes, J. C., and Neale, R. C., 1936, A new preparation protecting against rat liver necrosis. *Proc. Soc. exp. Biol., N.Y.*, XXXIV., 319.

Forbes, J. C., Neale, R. C., and Scherer, J. H., 1936, A liver preparation protecting against necrosis from chloroform or carbon tetrachloride administration. *J. Pharm. exp. Therap.*, LVIII., 402.

Hall, M. C., 1921, The use of carbon tetrachloride for the removal of hookworms. *J. Amer. med. Ass.*, LXXVII., 1641.

Hassan, A., and Salah, M., 1935, Investigation on carbon tetrachloride intoxication. *J. Egypt. med. Ass.*, XVIII., 207.

Hussameddin, 1931, Sur l'ankylostomiase répandue dans le département de Rizé en Turquie et sur la lutte entreprise contre cette maladie. *Bull. Off. int. Hyg. publ.*, XXIII., 1824

Klotz, J. D., 1934, Über den Wert des Tetrachlorkohlenstoffes als Anthelminthikum beim Hunde in Verbindung mit Kohle. *Dissertation. Königsberg*, 31 pp.

Lambert, S. M., 1933, Hookworm disease in the South Pacific. Ten years of tetrachlorides. *J. Amer. med. Ass.*, C., 247.

Lindsay, J. W., 1935, personal communication quoted by Lane, C. The appraisement of hookworm-killing drugs. *Lancet, i.*, 1459.

Maplestone, P. A., and Mukerji, A. K., 1929, Tetrachlorethylene in the treatment of hookworm disease. *Indian med. Gaz.*, LXVI., 424.

Nag, S. C., 1929, Notes on the use of carbon tetrachloride. *Ibid.*, LXIV., 683.

Oxer, D. T., 1933, Carbon tetrachloride—a note on its toxicity for sheep. *J. Coun. sci. industr. Res. Aust.*, VI., 279.

Savignoni, F., and Maria, G. de, 1933, Azione di alcuni antielmintici sulla madre e sul prodotto del concepimento. *Sperimentale*, LXXXVII., 557.

Soper, F. L., 1935, El problema de la fiebre amarilla en América. *Bol. Ofic. sanit. pan-amer.*, XIV., 204.

Tomb, J. W., and Helmy, M. M., 1933, The toxicity of carbon tetrachloride and its allied halogen compounds. *J. trop. Med.*, XXXVI., 265 and 336.

TETRACHLOROETHYLENE

In a search for other halogen derivatives of carbon which should be less toxic than carbon tetrachloride, but equally, if not more, effective against hookworms, Hall and Shillinger (1925 [1 & 2]), introduced tetrachloroethylene (C_2Cl_4), as a result of finding that it successfully removed hookworms from dogs in doses of from 0·2 to 0·3 c.c. per kilogram of body-weight. In comparison with carbon tetrachloride the disadvantages of tetrachloroethylene lie in its more pronounced taste and its greater anæsthetic action, whereby such symptoms as giddiness, nausea and vomiting are more likely to occur.

It is also relatively unstable, for even at room temperatures the drug may decompose, giving rise to phosgene, hydrochloric acid and chlorine. If, however, it is kept from the air in well-stoppered amber bottles it does not break down on standing. Lamson, Robbins and Ward (1929) and others have investigated the pharmacology and toxicity of tetrachloroethylene. The boiling point of tetrachloroethylene is 121°–122° C., that of carbon tetrachloride 77° C., consequently there is little danger of its causing asphyxiation if it should accidentally enter the lungs. In fact, it is very difficult to produce complete anæsthesia in dogs except by employing the cone method, while the introduction of 1 c.c. of tetrachloroethylene directly into the larynx causes in dogs nothing more than cough, dyspnœa, retching and vomiting. In normal dogs receiving as much as 10 c.c. per kilogram of body-weight, or fifty times the therapeutic dose, no absorption takes place from the intestines unless fat is present in the alimentary tract, when a certain amount of absorption may occur. This fact probably accounts for occasional cases of intoxication in dogs such as that recorded by Stewart (1934). Chronic alcoholics can be given tetrachloroethylene with much greater safety than carbon tetrachloride. Dogs and cats fed on diets deficient in calcium were not made more susceptible to tetrachloroethylene. The majority of observers have failed to produce in dogs anything more startling than slight fatty infiltration in the liver, while functional tests for both liver and kidneys are in no wise abnormal : no fall in blood sugar or change in blood guanidine is found even when dogs are kept on a diet low in calcium. The results of Christensen and Lynch (1933), who found lesions in the intestines, livers and hearts of dogs, are unconfirmed, nor have depression of the heart and respiration been observed in man. Maplestone and Mukerji (1933) recorded no changes in pulse or respiration, and only very slight rise of blood pressure after therapeutic doses in man.

The only animals in which pathological lesions have been recorded are cats, and then only when very large doses were administered. Even then no lesions were found by Maplestone and Chopra (1933) in the intestines and heart, but there were fatty changes in the liver, and in the kidneys evidence of a granular and tubular nephritis.

With therapeutic doses these lesions were absent. In cats and puppies the tendency to absorb tetrachloroethylene is greater than in adult dogs, but less in rabbits and mice, for these species pass into a state of narcosis when given amounts of 4 c.c. per kilogram of body-weight. Sheep and goats are particularly resistant, as Tunnicliff (1930) has given monthly doses for a year to many thousands of animals without a single death. According to Schlingman and Gruhzit (1927), chickens are very insusceptible and horses relatively susceptible to the drug.

Although thousands of persons have been treated with tetrachloroethylene no death has yet been attributed to its administration. This is in striking contrast to carbon tetrachloride. Kendrick (1929) observed one man who after 3 c.c. rapidly became unconscious and remained in this condition for three days, when quite suddenly he recovered consciousness and rapidly improved.

Unfortunately, evidence as to its anthelmintic efficiency is not very extensive, and some of the difficulties of interpreting such evidence as exists have already been discussed. Lambert (1933), for instance, reports 46,000 treatments in the Southern Pacific without a death or other untoward symptoms, and considers tetrachloroethylene safer than carbon tetrachloride, but is silent as to its anthelmintic effect.

Maplestone and Mukerji (1929) gave 3 c.c. to forty patients, with a cure rate, as estimated by direct centrifugal floatation, of only 15 per cent. When 1 c.c. of oil of chenopodium of unstated ascaridole content was added the cure rate among forty-two patients was 26·2 per cent. In 1933 they reported somewhat improved results, for among fifty cases one treatment produced thirty-one, or 62 per cent., of cures, two treatments thirty-seven, or 72 per cent. No explanation is forthcoming for the more satisfactory results obtained in the second experiment. Garrison (1934) gave children infected with *Necator americanus* 3 c.c. one day a week for three weeks, 1 c.c. being given at hourly intervals, followed by a purge. A brine-floatation method was used for stool examinations and at the end of four months only 7 per cent. were still harbouring parasites. Garin, Rousset and Gonthier (1932) gave 5 gm. doses daily for three consecutive days and obtained 82·5 per cent. of cures : thymol given in the same way cured only 47 per cent. Le Moult and Pirot (1934) " cured " eight out of eight persons with a single dose, but only four out of twenty-three with thymol and thirty-eight of fifty-two with oil of chenopodium.

As it has been found that tetrachloroethylene, when it does not expel worms, may temporarily inhibit the egg-laying capacities of the female, repeated doses are not recommended if critical comparative tests are to be made on the value of this anthelmintic. Tetrachloroethylene is, in fact, one of the few anthelmintics which merit massive controlled field investigations if its present claims to pre-eminence are to be fully established.

Against ascaris infection tetrachloroethylene would seem to be of little value and, like carbon tetrachloride, it may easily stimulate the migratory activities of the worms. Manson (1934) regards 4 c.c. of tetrachloroethylene and 1 c.c. of oil of chenopodium as the most satisfactory treatment for mixed helminthic infections in man.

Law (1933) claims that hookworms in silver foxes are readily treated by tetrachloroethylene, but as many as four doses may be necessary to ensure complete eradication. Rawson (1932) and Hanson (1932) have also obtained good results in the removal of nematodes from silver foxes.

Tetrachloroethylene may be given free, in gelatin capsules or as an emulsion consisting of 4 c.c. of tetrachloroethylene, well shaken up in magnesium sulphate solution. Lane (1935), however, states that tetrachloroethylene begins to separate out of such an emulsion ten seconds after ceasing to shake, and at the end of one minute it has all collected at the bottom of the flask.

References

CHRISTENSEN, B. V., and LYNCH, H. J., 1933, The effect of anthelmintics on the host. I. Tetrachlorethylene. II. Hexylresorcinol. *J. Pharmacol.*, XLI., 311.

GARIN, C., ROUSSET, J., and GONTHIER, B., 1932, Organisation de la lutte contre l'ankylostomose dans une exploitation minière : deuxième étape : le traitement et la prophylaxie. *Méd. du Trav.*, IV., 23.

GARRISON, H. F., 1934, Comparative study of value of tetrachlorethylene as means of ultimate control and eradication of hookworm disease in children. *Sth. med. J.*, XXVII., 24.

HALL, M. C., and SHILLINGER, J. E., 1925^1, Tetrachlorethylene, a new anthelmintic. *Amer. J. trop. Med.*, V., 229.

HALL, M. C., and SHILLINGER, J. E., 1925^2, Tetrachlorethylene, a new anthelmintic for worms in dogs. *N. Amer. Vet.*, VI., 41.

HANSON, K. B., 1932, Parasites of ranch foxes and their treatment. *J. Amer. vet. med. Ass.*, LXXX., 202.

KENDRICK, J. F., 1929, The treatment of hookworm disease with tetrachlorethylene. *Amer. J. trop. Med.*, IX., 483.

LAMBERT, S. M., 1933, Hookworm disease in the South Pacific : ten years of tetrachlorides. *J. Amer. med. Ass.*, C., 247.

LAMSON, P. D., ROBBINS, B. H., and WARD, C. B., 1929, The pharmacology and toxicity of tetrachlorethylene. *Amer. J. Hyg.*, IX., 430.

LAMSON, P. D., BROWN, W. H., and WARD, C. B., 1932, Anthelmintics : some therapeutic and practical considerations on their use. *J. Amer. med. Ass.*, XCIX., 292.

LANE, C., 1935, The appraisement of hookworm-killing drugs. *Lancet*, *i.*, 1459.

LAW, R. G., 1933, Hookworm infection in foxes. *Bull. Dept. Game and Fisheries, Ontario.*

LE MOULT and PIROT, 1934, Note sur quelques essais thérapeutiques dans l'ankylostomose. *Arch. Méd. Pharm. nav.*, CXXIV., 348.

MANSON, D., 1934, A comparative record of anthelmintic treatment with tetrachlorethylene and oil of chenopodium. *Indian med. Gaz.*, LXIX., 500.

MAPLESTONE, P. A., and CHOPRA, R. N., 1933, The toxicity of tetrachlorethylene to cats. *Ibid.*, LXVIII., 554.

MAPLESTONE, P. A., and MUKERJI, A. K., 1929, Tetrachlorethylene in liver rot of sheep. *Ibid.*, LXIV., 424.

MAPLESTONE P. A., and MUKERJI, A. K., 1933, Carbon tetrachlorethylene in the treatment of hookworm infection. *Ibid.*, XLVIII., 617.

RAWSON, G. W., 1932, A comparison of carbon tetrachloride and tetrachlorethylene. *J. Amer. vet. med. Ass.*, LXXX., 600.

SCHLINGMAN, A. S., and GRUHZIT, O. M., 1927, Studies on the toxicity of tetrachlorethylene, a new anthelmintic. *Ibid.*, LXXI., 189.

STEWART, M., 1934, Toxic symptoms following the administration of tetrachlorethylene to a dog. *Vet. Rec.*, XIV., 378.

TUNNICLIFF, E. A., 1930, Monthly treatment of goats with tetrachlorethylene. *J. Amer. vet. med. Ass.*, LVII., 338.

HALOGEN DERIVATIVES OF CARBON

Apart from carbon tetrachloride and tetrachloroethylene, the anthelmintic efficiency of a number of halogen derivatives of carbon has been tested. Chloroform, which long enjoyed a reputation in the treatment of hookworm disease, was found by Hall and Foster (1918) and Hall (1919) to have only a 50 per cent. efficiency in the removal of hookworms from the dog. In 1921 Hall published his results on carbon tetrachloride, and later Hall and Shillinger (1925) introduced tetrachloroethylene, which they regarded as probably a safer remedy than carbon tetrachloride. Hall and Shillinger (1924) also tested the anthelmintic effects of ethylene dichloride ($C_2H_4Cl_2$) : this substance was less efficient than either chloroform or carbon tetrachloride.

Hexachloroethane or carbon trichloride (C_2Cl_6) was also quite ineffective as an anthelmintic against hookworms in dogs (Hall and Cram, 1925), although de Blieck and Baudet (1928) and Hilz and Scheuble (1928) both employed it against liver flukes in sheep and cattle with satisfactory results, the drug being specially effective when injected intraperitoneally. Pentachloroethane ($CHCl_2.CCl_3$) and tetrachloroethane ($CHCl_2.CHCl_2$) had no action on liver flukes.

Daubney (1930) found pentachloroethane and tetrachloroethane exceedingly toxic to sheep, but hexachloroethane in a dose of 0·25 gm. per pound of body-weight removed 99·6 per cent. of the hookworms from one sheep, though smaller doses and other samples of the drug failed to produce such good results. Trichloroethylene ($CHCl = CCl_2$) was far too toxic for use in sheep.

The most extensive series of experiments on chlorinated alkyl hydrocarbons has been carried out by Wright and Schaffer (1932), who have attempted to correlate anthelmintic efficiency with the chemical constitution and physical properties of the following compounds : *n*-propyl chloride, *n*-butyl chloride, *n*-amyl chloride, *n*-hexyl chloride, 2-chlorobutane, 2-chloropentane, 3-chloropentane, tertiary amyl chloride, ethylidene chloride, *n*-butylidene chloride, propylene chloride, trimethylene chloride, amylene dichlorides, 1-2-3-trichloropropane, trichloroethane, *S*-tetrachloroethane, pentachloroethane, and trichloroethylene.

The correlation between anthelmintic efficiency, on the one hand, and chemical structure and physical properties on the other, was not in all series very clear. Thus, increasing the length of the hydrocarbon chain in normal monochloro-compounds did not lead to a progressive rise or fall in efficiency, for *n*-butyl chloride was the most efficient member, but *n*-hexyl chloride was more efficient than *n*-amyl chloride. Increasing the length of the hydrocarbon chain in secondary monochloro-compounds did, however, increase anthelmintic efficacy, for 2-chloropentane was nearly five times as efficient in removing hookworms from the dog

THE RELATIVE EFFICIENCY OF VARIOUS ANTHELMINTICS AGAINST
(Lamson and

	Hexylresorcinol	6-hexyl-*m*-cresol	*o*-heptylphenol
Pig Ascaris *in vitro*. Percentage killed in a given time.	100 in 2′ 90 in 1′ (1 in 1,000 suspension in saline).	100 in 30′ 90 in 20′ 60 in 10′ 40 in 5′ 10 in 2′ 10 in 1′ (1 in 1,000 suspension in saline).	100 in 30′ 90 in 20′ 65 in 10′ 10 in 2′ (1 in 1,000 suspension in saline).
Ascaris in dog. Percentage worms removed.	0·50 gm./kg. removed 100. 0·025 gm./kg. removed 70.	0·2 gm./kg. removed 97. 0·1 gm./kg. removed 68.	0·2 c.c./kg. removed 74. 0·1 c.c./kg. removed 46.
Ascaris in man. Percentage worms removed.	1 gm. removed 90 (cured 70 p.c.). 1 gm. removed 96 (cured 72 p.c.).	4·2 c.c. removed 49 (cured 5 p.c.).	4·2 c.c. removed 38 (cured 9 p.c.).
Toxicity to rats. Percentage killed.	0·35 gm./kg. killed 50.	2·3 c.c./kg. killed 50.	2·8 c.c./kg. killed 50.

as 2-chlorobutane. The increased efficacy is here associated with decreased water solubility. Similarly, increasing the length of the hydrocarbon chain in normal dichloro-hydrocarbons having two chlorine atoms attached to the end carbon atom, resulted in increased anthelmintic efficacy, the change in chemical composition being such as to bring the water solubility from a point above the optimum range to a position within the optimum range. The addition of chlorine to a carbon atom to which are attached one or more chlorine atoms does not invariably lead to increased anthelmintic efficacy, but when increased efficacy does occur there is a change in water solubility from a solubility above the range of optimum solubility to one within that range. Increase

PIG ASCARIS *IN VITRO*, ASCARIS IN DOGS AND ASCARIS IN MAN
Brown, 1936)

santonin	carbon tetrachloride	tetrachloro-ethylene	chenopodium oil	thymol
0 in 3 hrs. (1 in 1,000 suspension in saline).	10 in 3 hrs. (1 in 1,000 suspension in saline).	100 in 2 hrs. 80 in 1½ hrs. 10 in 1 hr. (1 in 1,000 suspension in saline).	100 in 30′ 85 in 20′ 60 in 10′ 70 in 5′ (1 in 1,000 suspension in saline).	100 in 30′ 90 in 20′ 20 in 10′ 10 in 2′ (1 in 1,000 suspension in saline).
0·025 gm./kg. removed 23. 0·013 gm./kg. removed 25 (2 doses removed 70).	0·3 c.c./kg. removed 100. 0·1 c.c./kg. removed 68.	0·2 c.c./kg. removed 100. 0·1 c.c /kg. removed 94.	0·1 c.c./kg. removed 100. 0·05 c.c./kg. removed 71.	0·100 gm./kg. removed 100. 0·050 gm./kg. removed 80.
0·32 gm. removed 87·3 (cured 80 p.c.). 0·32 gm. cured 92. 0·32 gm. cured 20–66.	1·5–3 c.c. removed 2·7 (cured 9·4 p.c.). 3 c.c. cured 22·2 p.c.	3 c.c. removed 35·6. 3 c.c. removed 0. 4 c.c. removed 0.	1·5 c.c. removed 69·8. 1·5 c.c. removed 83·2. 2 c.c. removed 94·9.	3 gm. removed 20. 4 gm. cured 18·6. 6 gm. removed 32·7.
1·9 c.c./kg. killed 50.	4 c.c./kg. killed 40.	4 c.c./kg. killed 40.	0·11 c.c. killed 50 p.c. (equivalent to 0·08 c.c. ascaridole).	0·27 gm./kg. killed 50.

in chlorine content of the molecule by the addition of chlorine atoms to different carbon atoms has also no constant bearing on anthelmintic efficacy, nor has a spreading of the chlorine atoms.

In compounds with the same number of carbon, hydrogen and chlorine atoms, changes in the position of the chlorine atom result in compounds showing marked differences in efficacy, water solubility being perhaps the most important factor. Increasing the chlorine content of the molecule was in some cases associated with an increase in anthelmintic efficacy when the compounds so formed fell within the range of water solubility between 1 : 1,250 and 1 : 5,300. This appears to be the range of optimum solubility, for compounds possessing solubilities from 1 : 115 to 1 : 1,000 show little or no anthelmintic efficiency against hookworms; in fact water solubility is the factor most definitely correlated with the anthelmintic efficacy of chlorinated alkyl hydrocarbons.

All compounds of this group, however, produce in greater or less degree degenerative changes in the gastro-intestinal tract, liver and kidneys of dogs: nevertheless, in the case of *n*-butyl chloride, 2-chloropentane, 3-chloropentane and *n*-butylidene chloride, the most promising of the compounds here discussed for the treatment of hookworm, the lesions are probably insufficient to militate against their use. *n*-Butyl chloride, *n*-propyl chloride and *n*-butylidene chloride were effective in the removal of ascarids from the dog and were well tolerated, but the only compound, pentachloroethane, which was efficacious against hookworms, ascarids and whipworms was extremely toxic for the host.

Substitution of other halogens for chlorine in the hydrocarbon molecule makes less difference to anthelmintic efficiency than does a change in water solubility. In addition, with compounds containing iodine and bromine, the most effective had solubilities of 1 : 1,000 to 1 : 1,700.

n-Propyl iodide was slightly more irritating to the gastro-intestinal tract than was *n*-propyl chloride; the former caused cystitis: *n*-butyl iodide and *n*-butyl bromide were also more irritant to the gastro-intestinal tract than *n*-butyl chloride. There was an entire absence of correlation between anthelmintic action on hookworms and ascarids.

In general, therefore, it may be said that in any one homologous series anthelmintic efficiency increases with the lengthening of the hydrocarbon chain. At the same time there is also a corresponding decrease in water solubility, with the result that a stage is reached when the solubility of a higher member of the series will be so slight that there will be little or no increase of anthelmintic efficiency. In each series there thus exists an optimum solubility range within which greatest anthelmintic efficiency exists. This range differs in each series of homologous compounds.

Solutions of these chlorinated hydrocarbons or of ascaridole or chenopodium oil, designed for administration as anthelmintics, have been made by dissolving them with the salts of basic derivatives of higher fatty acids, such as the benzoate or lactate of a 4-diethylaminoethoxyoleyl anilide (British Patent 429,498; 429,594/1935).

Very few of the chlorinated hydrocarbons have received extensive clinical trial, but Mönnig (1933) has tested the efficiency of *n*-butylidene chloride in horses. Doses of 0·2 to 0·3 c.c. per kilogram of body-weight, followed five hours later by linseed oil, was effective against *Strongylus*, while doses of 2 c.c. removed all ascaridia from poultry.

COMPARATIVE EFFICIENCY OF HALOGENATED ALKYL HYDROCARBONS

Compound	Water Solubility	Percentage of worms removed (doses 0·1–0·5 c.c per kgm.	
		Ascarids	Hookworms
n-Propyl chloride .	1 : 4,000	81·8	0
n-Propyl iodide .	1 : 1,000	80·4	73·7
n-Butyl chloride .	1 : 1,700	98·0	82·0
n-Butyl bromide .	1 : 1,600	15·0	84·8
n-Butyl iodide .	1 : 4,000	83·1	32·1
n-Amyl chloride .	1 : 5,000	33·3	10·3
n-Amyl iodide .	1 : 12,000	94·5	13·0

Physical Properties and Anthelmintic Efficiency (

Name	Structural formula.	Boiling point °C.
n-Propyl chloride	$CH_3.CH_2.CH_2Cl$	46–49
n-Butyl chloride	$CH_3.CH_2.CH_2.CH_2Cl$	77–79
n-Amyl chloride	$CH_3.CH_2.CH_2.CH_2.CH_2Cl$	105·5–107
n-Hexyl chloride	$CH_3.CH_2.CH_2.CH_2.CH_2.CH_2Cl$	134–136
2-chlorobutane	$CH_3.CH_2.CHCl.CH_3$	64–68
2-chloropentane	$CH_3.CH_2.CH_2.CHCl.CH_3$	97–99
3-chloropentane	$CH_3.CH_2.CHCl.CH_2.CH_3$	96·5–98·5
Tertiary amyl chloride	$CH_3.CH_2.CCl(CH_3)_2$	85–87
Ethylidene dichloride	$CH_3.CHCl_2$	57–57·5
n-Butylidene dichloride	$CH_3.CH_2.CH_2.CHCl_2$	115–116·5
Propylene dichloride	$CH_3.CHCl.CH_2Cl$	96·5–97
Trimethylene dichloride	$CH_2Cl.CH_2.CH_2Cl$	121–121·4
Amylene dichlorides	$\left\{\begin{array}{l} CH_3.CH_2.CH_2.CHCl.CH_2Cl \\ CH_3.CH_2.CHCl.CHCl.CH_3 \\ CH_3.CH_3.CCl.CHCl.CH_3 \end{array}\right\}$	142–160
1-2-3-trichloropropane	$CH_2Cl.CHCl.CH_2Cl$	158–161
Trichloroethane	$CH_2Cl.CHCl_2$	114–116
S-Tetrachloroethane	$CHCl_2.CHCl_2$	146·5–147
Pentachloroethane	$CHCl_2.CCl_3$	161–165
Trichloroethylene	$CHCl = CCl_2$	87–88

Certain chlorinated butanes are, it is claimed by Marcenac (1934), of considerable value in the treatment of cylicostomosis of the horse. To be effective in this disease the compound must be relatively insoluble, but active against all stages of the parasite. These conditions are fulfilled by 2 : 2-dichlorobutane and its unsaturated derivatives 2-chloro-butylene (MeCCl : CHMe) in a dose of 0·2 c.c. per kilogram of body-weight. Owing to their low solubilities the compounds require about six days for their complete removal.

References

Daubney, R., 1930, Miscellaneous experiments with anthelmintics chiefly alkyl-chlorides, in the treatment of nematode infestations of sheep. *Vet. J.*, **LXXXVI.**, 5.

De Blieck, L., and Baudet, E. A. R. F., 1928, Hexachlooraethaanals middel tegen distomatosis bij het rund. *Tijdschr. Diergeneesk. Utrecht*, **LV.**, 429.

Chlorinated Alkyl Hydrocarbons (Wright and Schaffer, 1932)

Specific gravity 25°/25°	Solubility at 25° C. amount of water necessary to dissolve 1 gm. of compound c.c.	Percentage of worms removed (doses 0·3–0·5 c.c. per kgm.)		
		Ascarids	Hookworms	Whipworms
0·885	400	100	0	1·2
0·875	1,700	98·4	89·1	28·0
0·874	5,000	33·3	10·4	38·4
0·876	12,000	35·1	25·0	16·7
0·870	1,000	71·4	22·2	0·4
0·864	4,000	77·2	92·6	2·2
0·866	4,000	56·4	100	63·1
0·860	300	0	0	1·1
1·175	180	None present.	2·8	0
1·084	2,000	100	97·0	51·0
1·170	340	75	37·1	12·9
1·170	350	47·6	0	0
1·000	3,500	94·3	80·5	0·6
1·395	420	—	—	—
1·453	270	75·0	0	0
1·589	350	100	0	0
1·677	2,900	100	96·2	95·7
1·456	550	97·0	16·0	21·1

Hall, M. C., 1919, Studies on anthelmintics. III. Chloroform as an anthelmintic. *J. Amer. vet. med. Ass.*, **LV.**, 652.

Hall, M. C., 1921, Carbon tetrachloride for the removal of parasitic worms, especially hookworms. *J. agric. Res. Washington*, **XXI.**, 157.

Hall, M. C., and Cram, E. B., 1925, Carbon trichloride as an anthelmintic and the relation of its solubility to anthelmintic efficacy. *Ibid.*, **XXX.**, 949.

Hall, M. C., and Foster, W. D., 1918, Efficacy of some anthelmintics. *Ibid.*, **XII.**, 397.

Hall, M. C., and Shillinger, J. E., 1924, Critical tests of miscellaneous anthelmintics. *Ibid.*, **XXIX.**, 313.

Hall, M. C., and Shillinger, J. E., 1925, Tetrachlorethylene, a new anthelmintic for worms in dogs. *N. Amer. Vet.*, **VI.**, 41.

Hilz, K., and Scheuble, R., 1928, Versuche zur Behandlung der Leberegelseuche bei Schafen und Rindern. I. Mitteilung: Die Wirkung von gechlorten Kohlenwasserstoffen der Methan- und Aethanreihe. *Münch. tierärztl. Wschr.*, **LXXIX.**, 401, 419.

Lamson, P. D., and Brown, H.W., 1936, Methods of testing the anthelmintic properties of ascaricides. *Amer. J. Hyg.*, **XXIII.**, 85.

Marcenac, M., 1934, Pouvoir anthelmintique de certains composés chlorés du butane dans la cylicostomose du cheval. *C. R. Acad. Sci., Paris*, **CXCVIII.**, 510.

MÖNNIG, H. O., 1933, Recent advances in the knowledge of anthelmintics. *J. S. Afr. vet. med. Ass.*, **IV.**, 206.

WRIGHT, W. H., BOZICEVICH, J., UNDERWOOD, P. C., and SCHAFFER, J. M., 1933, Butylidene chloride, a new anthelmintic. *Vet. Med.*, **XXVIII.**, 52.

WRIGHT, W. H., and SCHAFFER, J. M., 1929, Anthelmintic tests of chlorinated alkyl hydrocarbons and a correlation between the anthelmintic efficacy and the chemical composition. *J. Parasit.*, **XVI.**, 107.

WRIGHT, W. H., and SCHAFFER, J. M., 1931, The comparative anthelmintic efficacy for ascarids and hookworms in the dog of halogenated hydrocarbons containing chlorine, bromine and iodine. *Ibid.*, **XVIII.**, 44.

WRIGHT, W. H., and SCHAFFER, J. M., 1932, Critical anthelmintic tests of chlorinated alkyl hydrocarbons and a correlation between the anthelmintic efficacy, chemical structure and physical properties. *Amer. J. Hyg.*, **XVI.**, 325.

SCHISTOSOMIASIS

The number of persons suffering from schistosomiasis is probably second only to the number of those infected with hookworms, for schistosomiasis is found not only throughout Africa, but in parts of China, the Philippines, Formosa and in one of the Japanese islands, as well as in the northern parts of South America and many of the West Indies.

In the south of Portugal also there is an endemic focus of *Schistosoma hæmatobium.* In Egypt alone it is estimated that of a total population of fifteen million some ten million persons are infected with schistosomiasis.

In attempts to cure schistosomiasis innumerable drugs have been used, ranging from those recommended in the Ebers papyrus to synthetic chemicals such as arsphenamine; time seemed the only remedy till the introduction of tartar emetic and emetine so completely revolutionised treatment that a rapid cure can now be obtained in a considerable percentage of cases.

COMPOUNDS OF ANTIMONY

McDonagh (1915), in his treatise on venereal diseases, was the first to recommend the use of antimony in the treatment of vesical bilharziasis, but his patients do not appear to have been observed for a long enough period to establish a definite cure. Christopherson (1918), however, while injecting a patient with tartar emetic for kala-azar, noticed an improvement in the symptoms of urinary bilharziasis and thus independently re-discovered the curative action of antimony, successfully treating a series of cases of infection due to *Schistoma hæmatobium* at the Khartoum Civil Hospital. As a result of the publication of Christopherson's paper, McDonagh (1918) directed attention to the fact that in 1912 he had successfully treated twenty-three cases of bilharziasis, using not only tartar emetic, but also colloidal antimony and " antiluetin," a double salt of potassium antimonyl tartrate and ammonium tartrate, the results with the two latter drugs being better than those with tartar emetic. Wiley (1918) also reported that in 1916 he had cured a case of vesical bilharziasis with antimony at the Australian Dermatological Hospital in Cairo. In the last twenty years many millions of persons have been treated with antimony preparations, for in Egypt alone it is estimated that nearly a million persons seek treatment every year.

At first antimony salts of organic acids were exclusively used, later, with the introduction of "fouadin," complex trivalent, more correctly tervalent, antimony compounds of pyrocatechol have been largely employed.

Salts of Organic Acids. Potassium antimonyl tartrate, tartar emetic, $[C_4H_4O_7SbK]_2H_2O$, the salt most commonly used, contains 36·6 per cent. of tervalent antimony and is injected intravenously as a 6 per cent. solution, having a *p*H of 4·5. For an adult, twelve intravenous injections are given at the rate of one every other day. The first three injections consist of 0·5 c.c., 1 c.c. and 1·5 c.c.; thereafter 2 c.c. till a total of 24 c.c. has been injected. Provided that there are no signs of intolerance, that the disease is in an early stage, and that the patient completes the course, there is a strong probability that such treatment will ensure permanent cure. *Schistosoma mansoni* and *S. bovis* appear to be rather more resistant to tartar emetic than *S. hæmatobium*, but there is little evidence of the development of antimony resistant worms. Nevertheless, tartar emetic has many disadvantages, not least the length of time for which treatment must be continued: in Egypt quite 50 per cent. of patients fail to take the full course.

The fact that the injections must be given intravenously is also a serious drawback. Any escape of fluid into the perivascular tissue produces intense irritation which may go on to necrosis: local thrombosis from injury to the vessel wall is by no means rare. Solutions of tartar emetic must be freshly prepared, as prolonged standing is associated with increased toxicity.

Prolonged boiling, on the other hand, renders the solution opalescent and toxic, either as a result of dissociation or from the formation of oxides. McKenzie (1932) has recorded eleven cases of acute poisoning with four deaths resulting from intravenous injection of tartar emetic. The reactions which follow the injection of tartar emetic are:—

(1) Cough—in about 10 per cent. of cases, possibly due to minute embolisms in the lung capillaries, as the cough is associated with discomfort in the chest immediately after injection. Christopherson and Ward (1934) recommend the administration of omnopon (total alkaloids of opium), 0·25 grains, to relieve these symptoms.

(2) Nausea and vomiting occurs in 38 per cent. of cases: if persisting more than twenty-four hours it is an indication of antimony poisoning.

(3) Fever may be due to intercurrent infection. There is some evidence that antimony, like adrenalin, causes the spleen to contract, thus producing attacks in those infected with malaria.

(4) During the later part of a course of treatment there are not infrequently vague pains in the region of the liver. These pains are sometimes looked upon as due to direct action of antimony on the worms.

(5) Giddiness, collapse and sudden death occur in about 0·2 per cent. of cases. Recovery may sometimes follow the intracardiac injection of 0·2 c.c. of 1 in 1,000 adrenalin (Fakhry, 1931). In Egypt, according to Khalil (1935), there are about 2,000 deaths a year from antimony poisoning.

More remote complications of tartar emetic therapy are :—

(6) Labial herpes.

(7) Papular dermatitis.

Contra-indications to the use of tartar emetic are nephritis, heart failure and fever.

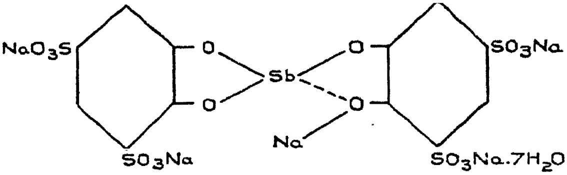
Owing to the disadvantages of potassium antimonyl tartrate numerous attempts have been made to find less toxic but equally efficient preparations of antimony.

Sodium antimonyl tartrate, $(C_4H_4O_7SbNa)_2H_2O$, is preferred by some, owing to its lesser toxicity and greater solubility. It is, however, less stable than the potassium salt, decomposes rather rapidly on boiling, and is quite as, if not more, painful than tartar emetic on injection: formerly it was more likely to contain impurities such as lead or arsenic, though a pharmacopœial scale preparation is now available for intravenous injection. The antimony content is slightly less than that of potassium antimonyl tartrate.

Fargher and Gray (1921) found that quinine antimonyl tartrate was five times less toxic to mice than tartar emetic, while lithium tartar emetic was two and a half times less toxic. Quinine antimonyl tartrate is a preparation impossible to administer except by mouth, when it is, of course, ineffective. Recently attention has been directed to the possible therapeutic activities of lithium antimonyl thiomalate ("Anthiomaline"), $[LiOOC.CH_2.CH(COOLi)S]_3Sb.9H_2O$: this preparation contains 16 per cent. of antimony and owing to its non-irritant action can be injected subcutaneously. It is quite stable in the dark and at temperatures below 80° C. For children under twelve years of age doses of 0·5 c.c. of a 6 per cent. solution are recommended to begin with, increasing to 1·5 c.c., while for adults doses should not exceed 4 c.c., otherwise salivation and nausea may occur. Cawston (1936 [1]) gives four injections a week, and states that a "permanent" cure can be obtained with less than 0·5 gm. of antimony. Piéri and Sardon (1936) gave doses of 3 c.c. twice a week to a child of twelve and obtained a cure after 30 c.c. had been administered. Montestruc and Bertrand (1936) found that a month after treatment nine patients had ceased to pass eggs. The drug was successfully given to patients where tartar emetic and fouadin had failed or had had to be abandoned as too toxic. Moulinard (1936), in a small series of boys aged from twelve to fourteen years, obtained 100 per cent. of cures at the end of the course, while some months later 85·7 per cent. were still negative : with tartar emetic 25 per cent. were negative and with emetine only 12·5 per cent. Moulinard states that there was a complete absence of local or general reactions, and that there are no contra-indications to the administration of this compound of antimony. Panayotatou (1936) gave eight to ten injections to twelve patients with urinary schistosomiasis, the doses being 1 c.c. for the first injection, 2 c.c. for the next three and thereafter 3 c.c. The injections were given intramuscularly and were well tolerated. Only dead eggs were seen after the fourth injection. Incidentally, Launoy (1934) has pointed out that cysteine in doses of 1 mgm. is capable of neutralising the toxic action of 0·1 mgm. of antimony as contained in lithium antimonyl thiomalate, if the two substances are injected simultaneously into mice. Further observations are obviously required before the true value of this compound in the treatment of schistosomiasis can be determined.

Pyrocatechol Compounds. An antimony compound, antimosan (von Heyden, 661), was introduced by the von Heyden Company and, though its exact nature was not disclosed, it was stated by Uhlenhuth, Kuhn and Schmidt (1924) to be a pyrocatechol derivative, probably an antimony compound of an alkali pyrocatechol-sulphonate or -carboxylate, in which the antimony is attached to the oxygen atoms of the two phenolic residues. It contains 12·5 per cent. of antimony in the tervalent form. Somewhat later another tervalent antimony compound (neo-antimosan) was prepared by Professor Hans Schmidt (1930), and under the name of " fouadin " was employed in Egypt by Khalil, Nazmi, Peter, el Din and el Betash (1929).

Fouadin, which contains 13·5 per cent. of antimony, is a tervalent antimony compound of pyrocatechol sodium disulphonate, the formula being given as :—



In a 7 per cent. solution this compound remains stable for as long as six months, even at the temperature of an Egyptian summer. The injections can be given intramuscularly in the gluteal region, there being very rarely any local reaction. Khalil (1935) reports only two abscesses in two thousand persons, involving 20,000 injections. As shown by X-ray examination, absorption is complete in six minutes.

The standard course now given in Egypt is as follows :—

1st day .	1·5 c.c. .	7 per cent. solution of fouadin.
2nd day .	3·5 c.c. .	7 ,, ,, ,, ,, ,,
3rd day .	5 c.c. .	7 ,, ,, ,, ,, ,,

and then the same dose of 5 c.c. every other day, or three times a week, to a total of 40 c.c. The maximum dose of 5 c.c. contains 42·5 mgm. of antimony. On the fifth to seventh day, after the fifth injection, the number of blackened granular eggs in the urine should be greatly increased, and by the end of the course, if not before, the eggs should all be dead.

The results obtained by Khalil (1935) in three years in Egypt are summarised in the table :—

Year	No. of cases	Percentage cured after			Percentage relapsed after 1 month
		9 injections	11 injections	13 injections	
1931	3,288	52·26	67·95	71·26	0·71
1932	2,299	62·20	82·70	86·80	9·30
1933	3,302	63·41	78·64	83·02	12·92

For unexplained reasons the percentage of relapses seems to have been much smaller in 1931 than in the two following years. In a later communication, Khalil (1936) states that of 1,938 cases 53 per cent. were cured after nine injections, 21 after eleven injections, and 10 per cent. after thirteen or more injections : 33 per cent. relapsed. Cawston (1937) in South Africa finds fouadin less efficient than tartar emetic.

Unfortunately, no exact evidence is available as to the relative efficiency of fouadin in treating infections due to *Schistosoma hæmatobium* and *S. mansoni*. There is, however, a general suggestion that infections caused by *S. mansoni* are less easily cured than those due to *S. hæmatobium.* Giovannola (1936), for instance, found that in rabbits experimentally infected with *S. mansoni* fouadin was inferior to potassium antimonyl tartrate. Montestruc and Bertrand (1936) also reported failures after treatment with fouadin.

In infections due to *S. japonicum*, fouadin, though it causes fewer reactions, is nevertheless undoubtedly less efficient than tartar emetic. Experimentally in rabbits, Lee (1932) found that though ova might temporarily disappear from the urine as the result of administration of fouadin there were many relapses.

Fouadin injected intravenously was less effective than when given intramuscularly. *In vitro*, Lee and Chung (1935), using a method described by Lee and Chu (1935) for keeping schistosome worms alive for as long as two and half months, found fouadin relatively ineffective, while clinically in man, Lee and Chung (1933), Li and Thompson (1934), Kan and Kung (1936) and others, have all noted temporary improvement, followed by a tendency to relapse, the percentage of permanent cures being less than that obtained with sodium or potassium antimonyl tartrate.

Toxic reactions due to fouadin are not so frequent as with tartar emetic, although nausea, epigastric pain, giddiness and vomiting have all been recorded. Bradycardia is common and is possibly of vagal origin, since it can be counteracted by atropine. Fever, œdema, abscess formation and herpes labialis are all rare, though in Egypt temporary exacerbation of a pellagrous erythema is not uncommon. In China ascites may be aggravated. Sudden death is very infrequent.

It has not infrequently been stated that antimony in therapeutic doses has a toxic action on the liver. This question has been investigated by Salah and Hassan (1935), who have found that neither fouadin nor tartar emetic deplete the liver of glycogen, nor do they cause any aggravation of parenchymatous jaundice. In cases of bilharzial cirrhosis, in fact, the liver functions were improved as a result of the administration of antimony. When given by mouth, fouadin is largely excreted in the fæces and is therapeutically inactive. When injected intramuscularly from 2·3 to 4·6 per cent. is found in the stools ; the remainder is excreted by the kidneys. Since the action of fouadin is cumulative, Khalil (1936) believes that many of the failures of fouadin are due to irregularities in the rate of excretion. In some persons fouadin is too rapidly excreted, such persons are resistant to treatment : in others excretion is very slow, such persons are liable to toxic symptoms and death. Only in persons with a mean rate of excretion does cure progress normally. Hassan (1937) has found that when antimony excretion in the urine is interfered with no increase occurs in the amount excreted in the fæces.

In an effort to provide a simple procedure to test the rate of excretion of fouadin, Khalil (1936) has proposed the following test :—

Shake together 5 c.c. of clear urine and 5 c.c. of a freshly prepared 0·05 per cent. solution of ferric chloride ($FeCl_3$), and add 1 c.c. of strong ammonia, again shaking. The colour, at first green, changes to violet and then red with the strong alkali.

In some persons the colour can be obtained fifteen minutes after an intramuscular injection of fouadin, in others not even after twenty-four hours, while in the normal subject the reaction comes on after three hours. The colour depends on the presence of pyrocatechol in the urine.

The intensity of the reaction is said to correspond roughly to the amount of antimony excreted, as judged by the stain produced on copper foil by Reinsch's test, but the test is in no way quantitative, and cases of antimony retention might well occur in the absence of pyrocatechol retention, or *vice versa.* Although the test may be of value in indicating certain cases that should not be given fouadin, more information would be obtained if it were possible to devise a simple quantitative test for the presence of antimony in the urine.

Concentrated fouadin was prepared in an effort to increase the amount of antimony in the fouadin molecule, 1 c.c. of the concentrated preparation containing 14·3 mgm. of tervalent antimony instead of 8·5 mgm. The salt is said to be a pyrocatechol disulphonate of calcium and sodium. Khaw (1934) found that " concentrated fouadin " given intramuscularly to rabbits infected with *Schistosoma japonicum* cured fourteen, killed five and left one uncured. In those that died the degree of fatty change in the livers was greater than that found in the untreated control rabbits. Results obtained by van Nitsen (1934) and Khalil (1935) with intramuscular injections show that though eggs disappear from the urine, abdominal pain and bloody stools are very frequent. Some success has been claimed with fouadin by various authors in the treatment of opisthorchiasis of the cat and also in China in cases of human infection with *Clonorchis sinensis.*

OTHER ANTIMONY COMPOUNDS

A series of compounds of antimony derived from hydroxyquinoline, originally prepared for their trypanocidal action, have been suggested as of possible value in the treatment of schistosomiasis. These compounds are :—

(1) Dn. 7 or "stibilase," a diethylamine antimonyl dihydroxyquinoline sulphonate.

(2) Dn. 18 or "tristibine," "antimonyl," sodium antimonyl-7-amino-8-hydroxyquinoline-sulphonate-*N*-methylene sulphite.

Dn. 7 contains 16·7 per cent. of tervalent antimony and is unstable and very hygroscopic. Dn. 18 contains 9·8 per cent. of tervalent antimony and is an amorphous, dark brown powder which is dissolved immediately before use. Both compounds can be injected subcutaneously, or intramuscularly.

Dn. 18 causes considerable pain at the site of injection. The results of extensive clinical trials have not yet been reported (*cf.* Cawston, 1936 [1]).

A number of compounds containing both arsenic and antimony were tested by Oesterlin (1934 [1]) for their action on mice and monkeys experimentally infected with *Schistosoma mansoni*. Sdt. 386 B, containing 18 per cent. of arsenic and 20 per cent. of antimony, was found effective in doses of 0·15 gm. per kilogram of body-weight. The same compound was also useful in the treatment of cats infected with *Opisthorchis felineus* (Oesterlin, 1934 [2]).

Pentavalent, more correctly quinquevalent, compounds of antimony, such as those of value in the treatment of kala-azar, are entirely inactive in the treatment of schistosomiasis, nor have the claims put forward for acridine derivatives been in any way made good. Emetine, which was first introduced in 1913 by Hutcheson in China and Tsykalas in Egypt, continues to be employed to a limited extent in cases where antimony salts are contra-indicated. Emetine hydrochloride is claimed by Kouri, Basnuevo and Fermoselle Bacardi (1935) to produce beneficial results in *Fasciola hepatica* infection after the administration of 0·03 gm. daily for seventeen or eighteen days. Its value in pulmonary distomiasis (paragonimiasis) is very doubtful.

In vitro experiments and observations on animals experimentally infected with schistosomes suggest that both emetine and antimony salts have a direct action on the adult worms. Kollath and Erhardt (1936) claim to have demonstrated a direct action on the part of fouadin by means of stains used for estimating variations in oxidation-reduction potential. No drug so far tested, including tartar emetic, has any prophylactic action on schistosome infections.

COPPER SALTS

Apart from antimony the action of only a few other metals has been tested in schistosomiasis. Van Nitsen (1937) claims, however, that copper compounds produce very definite therapeutic improvement. Preliminary observations were made with a 4 per cent. solution of ammoniacal copper sulphate, which was given intravenously in doses of from 5 to 10 c.c. to patients with intestinal schistosomiasis. More recently the action of two copper oxyquinoline derivatives have been investigated. These are :—

(1) "Dicuprène," stated to be diethylamine cuproxyquinoline disulphonate.

(2) "Paludex," stated to be sodium cuproxyquinoline sulphonate.

"Dicuprène" is a greenish powder containing 6·5 per cent. of copper and easily soluble in water, in which it dissolves to form a solution with a neutral reaction. It is given intravenously as a 10 per cent. solution. "Paludex" is also a green amorphous powder, containing 8·73 per cent. of the metal and freely soluble in water. It is given by mouth.

Thirty-five cases of intestinal schistosomiasis were treated with "dicuprène," the doses being from 5 to 10 c.c. given daily. In twelve patients with acute symptoms the diarrhœa disappeared in from one to four days, though from three to seven days were required for the disappearance of the ova. The largest total dose necessary to produce a "cure" was 70 c.c. In twenty-three chronic patients ova disappeared from the fæces three to nine days after the beginning of treatment, " cures " being recorded with total doses of from 20 to 70 c.c. The only accident that occurred with " dicuprène " was the onset of hæmaturia in one patient.

The dose of " paludex " varied from 0·6 gm. for a child weighing less than 10 kgm. to from 1·5 to 2 gm. for an adult. Eighteen patients with acute and ninety-seven with chronic symptoms were treated, ova disappearing in the case of the latter in from four to eighteen days. No toxic symptoms were noted, the treatment having in fact a tonic value. Unfortunately only a few patients were observed for as long as two months after treatment, so that no data are available in regard to the number of relapses.

It is obvious that so far the ideal anthelmintic for schistosomiasis has not been found : such an ideal drug must be active when given by mouth and must produce its curative effect after a comparatively short period. One difficulty in assessing the value of drugs in the chemotherapy of schistosomiasis lies in the difficulty of determining when a complete cure has been obtained. In order to determine whether a patient has been definitely cured, three tests have been suggested :—

(i.) The complement fixation test.
(ii.) The disappearance of eosinophilia.
(iii.) The microscopic appearance of degeneration in the ova.

The value of the complement fixation test requires further investigation, for, despite the absence of clinical evidence of the disease for a year, positive serological reactions may still be obtained. The persistence of intradermal tests requires further investigation.

Eosinophilia may sometimes increase during the course of treatment and may be due, not only to other helminthic infections, but to many other causes.

Microscopic examination of the ova in urine or fæces probably provides the best evidence of cure, though it is possible that female worms may be temporarily inhibited from laying eggs. Examinations must, therefore, be repeated at intervals over a considerable period ; in countries such as Egypt the chances of reinfection are considerable.

It is obvious that with the drugs at present in use chemotherapy alone cannot stamp out schistosomiasis. Hygienic measures to destroy the intermediate hosts must also be taken ; the provision of ducks in rural areas may be of value, provided they are not used by the inhabitants as an additional food supply. In using an emulsion of the berries of the tree *Balanites œgyptiaca*, as suggested by Archibald (1933), even if it could be provided in sufficient concentration in large masses of water, it must be remembered that not only molluscs but also tadpoles, frogs and fish would be destroyed, a serious interference with the œcological balance of nature.

References

Archibald, R. G., 1933, The use of the fruit of the tree *Balanites œgyptiaca* in the control of schistosomiasis in the Sudan. *Trans. R. Soc. trop. Med. Hyg.*, XXVII., 207.

Cawston, F. G., 1936 [1], The lithium salts of antimony in bilharzia infection. *Prescriber*, XXX., 233.

Cawston, F. G., 1936 [2], Recent research in the treatment of bilharzia disease. *J. trop. Med.*, XXXIX., 28.

Cawston, F. G., 1937, Recent progress in the cure of bilharzia disease in South Africa. *Parasitology*, XXIX., 1.

Christopherson, J. B., 1918, The successful use of antimony in bilharziosis administered as intravenous injections of antimonium tartaratum (tartar emetic). *Lancet*, *ii.*, 325.

Christopherson, J. B., and Ward, R. O., 1934, Bilharzia disease in England : the cystoscopic appearance of the bilharzia bladder before and after intravenous injections of sodium antimonyl tartrate. *Brit. J. Surg.*, XXI., 632.

Fakhry, A., 1931, Asphyxia following injection of tartar emetic. *Lancet*, *ii.*, 1325.

Fargher, R. G., and Gray, W. H., 1921, The chemotherapy of antimony : comparison of the antimonyl tartrates with the organic compounds of antimony. *J. Pharmacol.*, XVIII., 341.

Giovannola, A., 1936, Specific action of some drugs on experimental infections of *Schistosoma mansoni*. *Amer. J. Hyg.*, XXIV., 102.

Hassan, A., 1937, A quantitative study of the excretion of antimony. *J. trop. Med.*, L., 161.

Hutcheson, A. C., 1913, Results in thirteen cases of dysentery treated with emetine. *Chin. med. J.*, XXVII., 243.

Kan, H. C., and Kung, J. C., 1936, A preliminary report on specific treatment of Schistosomiasis japonica. *Chin. med. J.*, L., 1637.

Khalil, M., 1931, The specific treatment of human schistosomiasis (bilharziasis). *Beih. Arch. Schiffs- u. Tropenhyg.*, XXXV., 105.

Khalil, M., 1935, Chemotherapy of schistosomiasis. *J. Egypt. med. Ass.*, XVIII., 284.

KHALIL, M., 1936, Individual variation in the excretion of drugs as an important factor in their therapeutic results. A practical method for detecting the schistosomiasis cases with so-called idiosyncrasy to antimony to avoid fatalities and complications. *J. Egypt. med. Ass.*, XIX., 285.

KHALIL, M., NAZMI, M., PETER, F. M., EL DIN, M. S., and EL BETASH, M. L., 1929, Die Behandlung der Schistosomiasis mit intramuskulären "Fuadin" Injektionen. *Dtsch. med. Wschr.*, LV., 1125.

KHAW, O. K., 1934, Concentrated fouadin in the treatment of Schistosomiasis japonica in rabbits. *Proc. Soc. exp. Biol., N.Y.*, XXXII., 520.

KOLLATH, W., and ERHARDT, A., 1936. Lebensdauer, Redoxlage und Fuadinwirkung bei Opisthorchis *in vitro*. *Biochem. Z.*, CCLXXXVII., 287.

KOURI, P., BASNUEVO, J. G., and FERMOSELLE BACARDI, J., 1935, Poder fasciolicida del chlorhidrato de emetina. *Crón. méd.-quir.*, LX., 511.

LAUNOY, L., 1934, Action de la cystéine sur la toxicité de l'antimoine. *C. R. Acad. Sci., Paris*, CXCIX., 646.

LEE, C. U., 1932, Treatment of Schistosomiasis japonica with fouadin in experimentally infected rabbits. *Chin. med. J.*, XLVI., 1169.

LEE, C. U., and CHU, H. L., 1935. Simple technique for studying Schistosome worms *in vitro*. *Proc. Soc. exp. Biol. N.Y.*, XXXII., 1397.

LEE, C. U., and CHUNG, H. L., 1933, Treatment of Schistosomiasis japonica with fouadin in man. *Chin. med. J.*, XLVII., 1411.

LEE, C. U., and CHUNG, H. L., 1935, Action of various organic antimony compounds on *Schistosoma japonicum in vitro*. *Proc. Soc. exp. Biol. N.Y.*, XXXII., 1400.

LI, T. Y., and THOMPSON, H. G., 1934, Treatment of Schistosomiasis japonica with antimony compounds. Review of literature on Chinese cases. Report of 15 cases. *Trans. 9th Congr. Far East. Ass. trop. Med.* (Nanking), 2, 325.

MCDONAGH, J. E. R., 1915, "Biology and treatment of venereal diseases," London, p. 349.

MCDONAGH, J. E. R., 1918, Antimony in bilharziosis, *Lancet*, *ii.*, 371.

MCKENZIE, A., 1932, Fatalities following the administration of intravenous tartar emetic. *Trans. R. Soc. trop. Med. Hyg.*, XXV., 407.

MONTESTRUC, E., and BERTRAND, C., 1936, La bilharziose intestinale à la Martinique et son traitement par l'anthiomaline (antimoniothiomalate de lithium). *Rev. Méd. Hyg. trop.*, XXVIII., 31.

MOULINARD, M., 1936, Traitement de la bilharziose par l'anthiomaline. Résultats comparés à ceux obtenus avec l'émétine et l'émétique. *Ann. Méd. Pharm. colon.*, XXXIV., 352.

OESTERLIN, M., 1934 [1], Zur Chemotherapie der experimentellen Schistosomiasis. *Arch. Schiffs- u. Tropenhyg.*, XXXVIII., 433.

OESTERLIN, M., 1934 [2], Zur Chemotherapie des Katzen-Leberegels (*Opisthorchis felineus*). *Ibid.*, XXXVIII., 441.

PANAYOTATOU, A., 1936, Recherches sur l'action de l'antimoniothiomalate de lithium dans la bilharziose vésicale à Alexandrie d'Egypte. *Rev. Méd. Hyg. trop.*, XXVIII., 322.

PIERI, J., and SARDON, 1936, A propos d'un cas de bilharziose vésicale traité par le 110L (anthimoniothiomalate de lithium). *Bull. Soc. Path. exot.*, XXIX., 508.

SALAH, M., and HASSAN, A., 1935, The action of antimony on the liver with special reference to its use in the treatment of schistosomiasis. *Arch. Schiffs- u. Tropenhyg.*, XXXIX., 1.

SCHMIDT, H., 1930, Antimon in der Arzneimittelsynthese. *Z. angew. Chem.*, XLIII., 963.

TSYKALAS, D., 1913, Un cas de bilharz urinaire traité par l'émétine. *C. R. sect. méd. du Syll. Sc. Hell. d'Alexandrie*, VI., 13.

UHLENHUTH, H., KUHN, P., and SCHMIDT, H., 1924, Ueber ein neues trypanozides Antimonkomplexsalz (Heyden 661). *Dtsch. med. Wschr.*, L., 1288.

VAN NITSEN, R., 1934, Traitement de la bilharziose intestinale par la fouadine concentrée. *Bull. méd. Katanga*, XI., 123.

VAN NITSEN, R., 1937, Essais de traitement de la bilharziose intestinale par les sels de cuivre. *Ann. Soc. belge Méd. trop.*, XVII., 77.

WILEY, C. J., 1918, The treatment of bilharziosis by intravenous injections of tartar emetic. *Brit. Med. J.*, *i.*, 137.

FILARIASIS

Despite the severity of the lesions produced by filarial infection, no chemotherapeutic drug has yet been found to have any effect on the disease in man. Unfortunately, the filarial worms parasitic in man cannot be transmitted to laboratory animals.

The effects of a number of drugs have, however, been tested on the filarial worms of the dog, *Dirofilaria immitis*, which are found in the right auricle and ventricle of the heart and in the large blood vessels connecting therewith. Gentian violet, trypan blue, plasmoquin, bismuth, gold compounds, various arsenical preparations, oil of chenopodium, hexylresorcinol, mercurochrome, emetine and various quinquevalent antimony compounds, have no action in destroying either the microfilaria in the blood or the adult worms in the heart (Johnstone, 1936). Itagaki and Makino (1927), however, found that intravenous injections of sodium antimonyl tartrate caused a disappearance of microfilaria from the peripheral circulation of dogs, but the worms were not affected. Khaw and Cheu (1936), with antimosan and fouadin, succeeded in killing some of the adult worms, while the females which remained alive were sterilized. Thromboses and infarcts were not infrequently caused by dead or debilitated worms, and in curative doses the drug caused a considerable degree of fatty degeneration in all the internal organs. Popescu (1933) believes that cure can only be obtained with toxic doses, ulceration of the scrotum being an invariable sign of therapeutic efficiency. Nevertheless, Wright and Underwood (1936) believe that in countries where dogs are almost invariably incapacitated by filarial infection, fouadin is of considerable value. Of 997 infected dogs 90·3 per cent. were restored to usefulness by one or more courses of fouadin, and in only twenty-two was it found impossible to banish microfilaria from the peripheral blood-stream. The mortality was 4·9 per cent. Cheu and Khaw (1935) used concentrated fouadin in the treatment of dogs, and again removed microfilaria from the peripheral blood-stream without killing the adult worms. In eleven cases of human filariasis Khaw and Cheu (1936) unfortunately found fouadin entirely without effect.

In Indian cattle *Parafilaria multipapillosa* frequently causes slightly raised subcutaneous nodules which break down with hæmorrhage. Intravenous injections of 100 c.c. of 1 per cent. potassium antimonyl tartrate are said by Gulati (1934) to have a curative action.

References

CHEU, S. H., and KHAW, O. K., 1935, Treatment of *Dirofilariasis immitis* with concentrated fouadin. *Proc. Soc. exp. Biol N.Y.*, **XXXII.**, 1394.

GULATI, R. L., 1934, *Filaria hæmorrhagica* in cattle and its treatment. *Indian vet. J.*, **XI.**, 32.

ITAGAKI, S., and MAKINO, R., 1927, Studies on the treatment of *Microfilaria immitis* infection by intravenous injection of natrium antimonyl tartrate. *J. Japan. Soc. vet. Sci.*, **VI.**, 15.

JOHNSTONE, H. G., 1936, The chemotherapy of *Dirofilaria immitis*. *Amer. J. trop. Med.*, **XVI.**, 207.

KHAW, O. K., and CHEU, S. H., 1936, The treatment of dog heartworm (*Dirofilaria immitis* Leidy, 1856) with some organic antimony compounds. *Chin. med. J.*, Suppl. No. 1, 402.

POPESCU, F., 1933, L'action thérapeutique de la fouadine sur la filariose canine. *C. R. Soc. Biol. Paris*, **CXIV.**, 219.

WRIGHT, W. H., and UNDERWOOD, P. C., 1936, A survey of 1,000 dog heartworm cases treated with fouadin. *N .Amer. Vet.*, **XVII.**, 39.

CHAPTER II

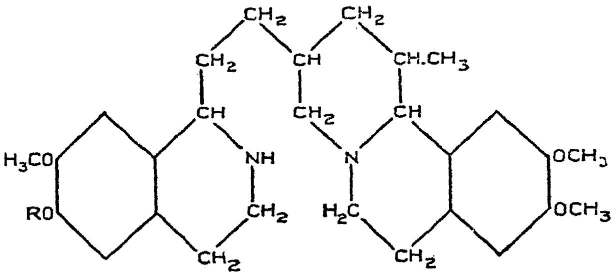
THE CHEMOTHERAPY OF AMŒBIASIS

DURING the war the treatment of amœbiasis with emetine hydrochloride showed that in a considerable percentage of cases this drug fails to eradicate parasitic amœbæ from the tissues, while its beneficial effects are often more than counterbalanced by its toxicity. These conclusions have been strongly reinforced by the results obtained during the 1933 epidemic of amœbiasis in the United States of America, an outbreak which incidentally proved conclusively that amœbiasis is a disease not necessarily confined to tropical climates. Numerous attempts have therefore been made to find substances which, while less toxic, are as efficient as emetine. As a result, amœbicidal action has been found among (1) quinoline derivatives, (2) kurchi alkaloids, (3) quinquevalent arsenicals, and (4) a heterogeneous collection of drugs as diverse as iodoform and hexylresorcinol.

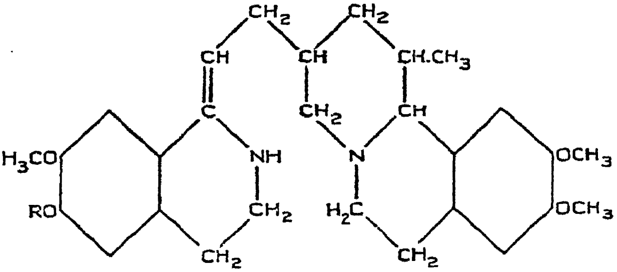
The ideal amœbicidal compound should rapidly destroy parasitic amœbæ both in the intestines and in other tissues. It should be capable of being given by mouth and should be readily absorbed from the intestine. Finally, it should be non-irritant and non-toxic, even after prolonged administration.

THE ALKALOIDS OF IPECACUANHA

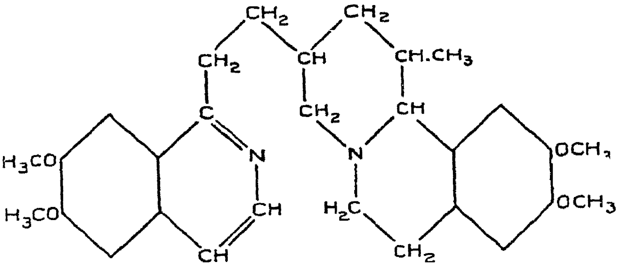
The medicinal qualities of ipecacuanha root were apparently known before the Spanish conquest to the natives of Brazil, whence it was introduced to Europe by Guillaume le Pois (Guilielmus Piso, 1658). A few years later it was being used in India. Helvetius successfully employed ipecacuanha in the treatment of the Dauphin son of Louis XIV, while Dr. Dover (1660–1742) included it in his famous powder. The roots of *Cephaëlis ipecacuanha* (Brot.) A. Rich constitute the Brazilian ipecacuanha of commerce, and probably also that obtained from Johore in Malaya. Not less than two-thirds of the total alkaloids should be non-phenolic. Brazilian ipecacuanha has 72 per cent. of its alkaloids in the form of emetine :



Cephaeline (R = H) and emetine (R = CH_3).



O-Methylpsychotrine (R = CH_3) and psychotrine (R = H).



Emetamine.

Indian ipecacuanha 70 per cent. (Chopra and Mukherjee, 1932). A second commercial variety known as Carthagena ipecacuanha is believed to be derived from *C. acuminata,* which flourishes in Colombia. The alkaloid emetine, though first isolated by Pelletier and Magendie (1817), was only obtained in a pure state by Paul and Cownley (1894), who separated from commercial emetine first the phenolic base cephaeline and later a third alkaloid psychotrine. To these Pyman (1917) added emetamine and *o*-methylpsychotrine. The structural formulæ of the ipecacuanha alkaloids have been elucidated by Brindley and Pyman (1927).

THE PHARMACOLOGY OF EMETINE

Tull Walsh (1891) appears to have been the first to administer, in place of ipecacuanha, the alkaloid emetine by mouth, in the form of the double iodide of emetine and mercury, and thus to prove that the curative action of ipecacuanha in amœbic dysentery is due to its alkaloidal content. More than twenty years later Rogers (1912) showed that salts of emetine introduced parenterally could successfully replace the oral administration of ipecacuanha root, while the other alkaloids obtained from ipecacuanha had little if any curative action in amœbic dysentery. The pharmacology and toxicity of emetine have naturally therefore been more extensively studied than those of the other ipecacuanha alkaloids.

Emetine, when directly applied to a mucous surface such as the tongue, has, according to Chopra, Gupta and Pillai (1927), an irritant action. The flow of saliva is increased, but the amount of the starch-digesting ferment is decreased. Peptic digestion, on the other hand, is stimulated by dilutions higher than 1 in 2,000. When given intravenously the tone and movement of the alimentary tract are stimulated as a result of a direct action of the alkaloid on the musculature of the tract, the nervous mechanism being unaffected; in large doses the intestinal musculature is relaxed and depressed (Epstein, 1932). In addition, there is increased vascularity of the gut, and both the volume and automatic movements of the spleen are augmented. This pooling of blood in the splanchnic vessels, the area chiefly affected by *Entamœba histolytica*, possibly helps the amœbicidal action of emetine. Chopra, Gupta and Roy (1935) find that the intravenous injection of therapeutic doses of emetine hydrochloride (1 mgm. per kilogram) into Belgian hares causes a decrease in the iodine content of the thyroid and a fall in the adrenaline content of the adrenals. This latter effect may in part explain the fall in blood pressure recorded by Tournade, Sarrouy and Curtillet (1935). Both emetine and cephaeline produce marked dilatation of the coronary vessels (Epstein, 1932). Since the toxic dose of emetine is very much the same on both oral and subcutaneous administration, absorption from the gut is probably complete. On the other hand, in man at least, emetine on subcutaneous injection is first partly excreted into the lumen of the intestines, but, as Mattei (1920) and others have shown, both in man and in animals it ultimately appears in the urine. Excretion is slow, continuing for days or even weeks, while the rate is by no means uniform. The experiments of Eggleston and Hatcher (1915) suggest that the emetic effects of emetine are due both to reflex and central stimulation. In therapeutic doses the musculature of the uterus is unaffected, so that the administration of emetine does not increase liability to abortion.

THE TOXICITY OF EMETINE

Emetine is a general cytoplasmic poison, and its effects are cumulative, a fact originally demonstrated by Dale (1915) in cats, and since found to apply both to man and to other animals. In guinea-pigs emetine has a cumulative action when given in repeated doses as small as 1/20 M.L.D. (Rosen, Martin and David, 1935). Both in animals and man there is, however, a wide range in individual susceptibility, though the liability to poisoning is undoubtedly increased if injury is already present to the parenchymatous cells of liver or kidney, the intestinal or cardiac musculature.

The toxicity of emetine itself, various emetine preparations and other ipecacuanha alkaloids for the monkey, rat, rabbit and cat, is shown in the table. As Anderson and Leake (1930) point out, the "toxic range" of emetine for all the larger mammals is relatively constant, and is in the neighbourhood of 15 to 20 mgm. per kilogram of body weight.

Since in man also toxic results have been noted with doses of 25 mgm. per kilogram of body weight, it is probably inadvisable to give human beings single doses of more than 1 mgm. per kilogram of body weight or a total of 10 mgm. per kilogram. Vedder (1914) recommended that the average adult dose should not exceed 0·65 gm.

A number of observers, Dale and Dobell (1917), Chopra, Ghosh and De (1924), Anderson and Leake (1930), Rhinehart and Anderson (1931), have investigated the histological changes produced by toxic doses of emetine. As a result of six or seven injections lesions are found in rabbits, especially in the heart muscle, which may show cloudy swelling, loss of transverse striation, hyaline degeneration and areas of fibrosis : hyaline change may also be found in the diaphragm. After eight or nine injections the lungs show œdema, congestion and hæmorrhage into the alveoli : widespread hæmorrhages from injury to the capillary endothelium are by no means rare.

After more prolonged administration lesions are found in the intestine, in the tubular epithelium of the kidneys and in the liver parenchyma. Similar lesions in man have been described by Bais (1921), Soca (1922), Leibly (1930) and others.

The clinical symptoms in man are in accord with the lesions described above. Loss of appetite, nausea, vomiting, vague abdominal pains and diarrhœa may occur after from four to six injections. More alarming symptoms are albuminuria, generalized œdema, petechial hæmorrhage, purpuric or urticarial skin rashes with pruriginous spots, hæmoptysis and signs of pulmonary or cerebral œdema (Chopra, 1934). Syncope and sudden death from heart failure are not unknown, while the rapid emetine pulse may be associated, as Chopra and Sen (1934) have pointed out, with changes in the electro-cardiogram indicative of myocardial damage. Clinically an increase in pulse rate with lowered blood pressure, sometimes associated with cardiac arhythmia, may be taken as an early indication of cardiac dysfunction, while globus hystericus and a feeling of constriction about the throat and chest have been observed. In subacute or chronic poisoning death is usually due to respiratory failure, cephaeline being approximately one and a half times as toxic as emetine. Jacovidès (1923) has described disordered vision associated with photophobia, amblyopia and central scotoma. The symptoms, often put down to neuritis, are possibly in part due to involvement of the peripheral nerves, for Young and Tudhope (1926) recorded Wallerian degeneration of nerve fibres, more especially at the distal ends.

References

ANDERSON, H. H., and LEAKE, C. D., 1930, The oral toxicity of emetine hydrochloride and certain related compounds in rabbits and cats. *Amer. J. trop. Med.*, **X.**, 249.

BAIS, W. J., 1921, Dood door emetine. *Geneesk. Tijdschr. Ned.-Ind.*, **LXI.**, 500.

BRINDLEY, W. H., and PYMAN, F. L., 1927, The alkaloids of ipecacuanha. Part IV. *J. Chem. Soc.*, **I.**, 1067.

CHEN, M. Y., and ANDERSON, H. H., 1930, Effect of emetine hydrochloride by subcutaneous injection on oxygen consumption in human subjects. *Proc. Soc. exp. Biol. N.Y.*, **XXVII.**, 521.

CHOPRA, R. N., 1934, The toxic effects of emetine. *Indian med. Gaz.*, **LXIX.**, 309.

CHOPRA, R. N., GHOSH, B. N., and DE, P., 1924, Some observations on the toxicity of emetine. *Indian med. Gaz.*, **LIX.**, 338.

CHOPRA, R. N., GUPTA, J. C., and PILLAI, K. V., 1927, Observations on the action of emetine on the gastro-intestinal tract. *Indian J. med. Res.*, **XV.**, 883.

CHOPRA, R. N., GUPTA, J. C., and ROY, A. C., 1935, The effect of emetine on the activity of the adrenal and thyroid glands. *Indian J. med. Res.*, **XXII.**, 771.

CHOPRA, R. N., and MUKHERJEE, B., 1932, Indian ipecacuanha. *Indian med. Gaz.*, **LXVII.**, 85.

CHOPRA, R. N., and SEN, B., 1934, Toxic effects of emetine on the cardiovascular system. *Indian med. Gaz.*, **LXIX.**, 262.

DALE, H. H., 1915, Preliminary note on chronic poisoning by emetine. *Brit. Med. J.*, *ii.*, 895.

DALE, H. H., and DOBELL, C., 1917, Experiments on the therapeutics of amœbic dysentery. *J. Pharmacol.*, **X.**, 339.

DALIMIER, R., 1917, La toxicité du chlorhydrate d'émétine. *Pr. méd.*, **XXV.**, 33.

EGGLESTON, C., and HATCHER, R. A., 1915, The seat of the emetic action of various drugs. *J. Pharmacol.*, **VII.**, 225.

EPSTEIN, D., 1932, The actions of emetine and cephaeline on the circulation, uterus and intestine. *Quart. J. Pharm.*, **V.**, 21.

JACOVIDÈS, 1923, Troubles oculaires à la suite d'injection d'émétine en 1916. *Pr. méd.*, **XXXI.**, ii., 979.

KOLMER, J. A., and SMITH, A. J., 1916, The bactericidal and protozoacidal activity of emetin hydrochloride *in vitro*. *J. infect. Dis.*, **XVIII.**, 266.

KOLMER, J. A., and SMITH, A. J., 1916, The bactericidal and protozoacidal activity of emetin hydrochloride *in vivo*. *Ibid.*, **XVIII.**, 274.

Lake, G. C., 1918, On the toxicity of emetine hydrochloride, with special reference to the comparative toxicity of various market preparations. *Bull. U. S. Hyg. Lab.*, **II.**, 41.

Leibly, F. J., 1930, Fatal emetine poisoning due to cumulative action. *Amer. J. med. Sci.*, **CLXXIX.**, 834.

Levy, R. L., and Rowntree, L. G., 1916, Toxicity of various commercial preparations of emetine. *J. Pharmacol.*, **VIII.**, 120.

Mattei, C., 1920, Toxicité, élimination urinaire et accumulation de l'émétine chez l'homme. *Bull. Soc. méd. Hôp. Paris*, **XXVI.**, 531.

Paul, B. M., and Cownley, A. J., 1894, The alkaloids of ipecacuanha. *Pharm. J.*, **XXV.**, 111, 373, 641, 690.

Pelletier and Magendie, 1817, Recherches chimiques et physiologiques sur l'ipécacuanha. *Ann. Chim.* (*Phys.*), **IV.**, 172.

Piso, G., 1658, De Indiae utriusque re naturali et medica. Amsterdam.

Pyman, F. L., 1917, The alkaloids of ipecacuanha. Part II. *J. chem. Soc.*, **CXI.** (i.), 419.

Rhinehart, J. F., and Anderson, H. H., 1931, Effect of emetine on cardiac muscle. *Arch. Path.*, **XI.**, 546.

Rogers, L., 1912, The rapid cure of amœbic dysentery and hepatitis by hypodermic injections of soluble salts of emetine. *Brit. Med. J.*, *i.*, 1424.

Rogers, L., 1914, The emetine and other treatment of amœbic dysentery and hepatitis including liver abscess. *Indian med. Gaz.*, **XLIX.**, 85.

Rosen, E. A., Martin, R. R., and David, N. A., 1935, Cumulative toxicity of emetine hydrochloride in guinea-pigs. *Proc. Soc. exp. Biol. N. Y.*, **XXXIII.**, 289.

Soca, F., 1922, A propos d'un cas de polynévrite émétique suivie de mort. *Bull. Soc. méd. Hôp. Paris*, **XXXVIII.**, 768.

Tournade, A., Sarrouy, C., and Curtillet, A., 1935, De l'action de l'émétine en injection intraveineuse sur les appareils vaso-moteurs et adrénalino-sécréteurs. *C. R. Soc. Biol.*, **CXIX.**, 237.

Vedder, E. B., 1914, Origin and status of emetine treatment of amœbic dysentery. *J. Amer. med. Ass.*, **LXII.**, 501.

Walsh, J. H. T., 1891, What is the rational treatment of amœbic dysentery? *Indian med. Gaz.*, **XXVI.**, 269.

Walters, A. L., and Koch, E. W., 1917, Pharmacological studies of the ipecac alkaloids and some synthetic derivatives of cephaeline. *J. Pharmacol.*, **X.**, 73.

Young, W. A., and Tudhope, C. R., 1926, The pathology of prolonged emetine administration. *Trans. R. Soc. trop. Med. Hyg.*, **XX.**, 93.

EMETINE HYDROCHLORIDE

Emetine hydrochloride is still the most commonly used salt of emetine, but in view of what has been said in regard to its toxicity, and more especially its cumulative action, it is doubtful whether its continued use can be justified. Nevertheless, in amœbic hepatitis and in actual liver abscess it is still the only drug that has a definite amœbicidal action. Only time can show whether or not the use of the newer amœbicides will increase the number of cases of amœbic hepatitis.

EMETINE AND BISMUTH IODIDE

This compound, a complex iodide of emetine and bismuth, contains not less than 25 per cent. and not more than 28 per cent. of emetine, and not less than 18 per cent. and not more than 21 per cent. of Bi ; under the action of the hydrochloric acid of the stomach, as pointed out by Martindale (1923[1]), it is slowly decomposed with the formation of bismuth oxychloride. The great drawback to the use of this compound is the intense nausea and vomiting to which it so frequently gives rise and the restricted diet which its administration necessitates, often with attendant mental depression. Manson-Bahr (1931) and others prefer to combine emetine and bismuth iodide with sodium iodohydroxy-quinoline sulphonate (chiniofon, p. 70), given per rectum, for certain cases have relapsed after as many as nine courses of emetine and bismuth iodide. Three cases of liver abscess are reported by Manson-Bahr after treatment confined to this drug.

OTHER EMETINE PREPARATIONS

(*a*) **Emetine periodide** ($C_{29}H_{40}N_2O_4 . 6I_2$), prepared by Martindale (1923[2]), is a dark purple crystalline powder containing 38·7 per cent. emetine and 61·3 per cent. iodine. It is now little used, since it appears to be less efficient than emetine and bismuth iodide.

(*b*) **Auremetine,** a combination of the periodides of emetine and auramine (tetramethyldiamino-diphenyl-ketoniminehydrochloride), has also been but little used.

(*c*) **Emetine antimony iodide,** an orange-yellow powder containing 33·5 per cent. of emetine and 13·7 per cent. of antimony. It does not appear to have been extensively used in the treatment of amœbiasis. Reed (1930), however, used it in four cases, which received total doses of from 33 to 65 mgm. in capsules heavily coated with salol (phenyl salicylate). The amœbæ were not eliminated and all the patients complained of nausea, vomiting and epigastric distress.

(*d*) **Emetine camphorsulphonate.** In order to overcome the depressant action of emetine, Beretervide and Grau (1935) have suggested the use of a compound of the emetine base of Reychler's camphor-β-sulphonic acid $(C_{10}H_{15}OSO_3H)_2$, which is said to be only one-third as toxic for animals as emetine. Two cases were treated for twenty days with 6 cgm. daily without any toxic symptoms, but the results on the amœbæ were not very pronounced.

(*e*) **Alkresta ipecac.** Another derivative of ipecacuanha, known as "Alkresta ipecac," is an adsorption product of the total alkaloids of ipecacuanha on hydrated aluminium silicate or Fuller's earth. The advantage claimed for this material is that it has no irritant effect on the gastric mucosa; larger quantities of emetine can therefore be introduced by mouth and a higher concentration obtained. Although its administration is well tolerated and is not followed by diarrhœa and vomiting, as is the case with emetine bismuth iodide, it has little effect, according to Chopra, Sen and Sen (1934), in eliminating amœbæ from the gut.

It is now generally agreed that in the acute stage of an early case of amœbic dysentery emetine has an excellent chance of curing the disease, the hyperæmia of the gut helping to ensure that any emetine circulating in the blood will have ready access to the parasites. In chronic cases emetine frequently fails to produce a permanent cure. This may be due to several factors :

(1) Too rapid excretion of the drug.

(2) Excystation of resistant cysts after cessation of treatment.

(3) Acquired resistance to emetine. Different strains of *Entamœba histolytica* vary in their resistance to emetine, while, according to Halawani (1930), partially resistant strains may be produced by exposing them for long periods to gradually increasing concentrations of the drug. St. John (1933) failed, however, to confirm these results.

(4) Failure of emetine to reach the amœbæ owing to fibrosis and thickening of the intestinal wall.

(5) Physical factors such as *p*H : in intestinal amœbiasis the *p*H of the gut may shift to the acid side of neutrality. It is possible that changes in the intestinal flora may effect the survival of amœbæ. (Knowles, Napier and Das Gupta, 1923).

GAVANO

The exact constitution of this compound has not yet been disclosed, although it is suggested by Chopra *et al.* (1934) that it is either a derivative of cephaeline or cephaeline itself. Each tablet contains 0·25 gm. of the active product. It is said by Fitzgerald (1933) to be far less toxic then emetine, and in cats it has a chemotherapeutic index of 1 : 3 ; there is no cumulative action, while through its action on the medullary centres it is said to increase vagal activity and thus stimulate intestinal peristalsis. Fitzgerald treated five cases, including himself, with satisfactory results. Two or three tablets a day were given by mouth for from seven to nine days to eighteen persons by Chopra, Sen and Sen (1934), the majority of whom were suffering from chronic amœbic dysentery. Six patients were cured, in ten the drug failed and in two others the results were indeterminate. The only visible ill-effects produced by the drug were a feeling of discomfort, sometimes amounting to pain, in the epigastric region, flatulence, heaviness in the head and occasionally a feeling of drowsiness. Nausea, vomiting and diarrhœa were not seen, but in two patients slight albuminuria developed. Akashi (1935) claims to have obtained rapid improvement in ten acute cases.

References

AKASHI, K., 1935, The treatment of amœbiasis with Gavano. *Taiwan Igakkai Zasshi*, **XXXIV.**, 189.

ANDERSON, H. H., and LEAKE, C. D., 1930, The oral toxicity of emetine hydrochloride and certain related compounds in rabbits and cats. *Amer. J. trop. Med.*, **X.**, 249.

BERETERVIDE, J. J., and GRAU, C. A., 1935, Una nueva sal de emetina : el canfo-sulfonato de emetina. *Prensa méd. Argentina*, **XXII.**, 671.

CHOPRA, R. N., SEN, S., and SEN, B., 1934, Treatment of chronic intestinal amœbiasis with Gavano, a derivative of ipecacuanha. *Indian med. Gaz.*, **LXIX.**, 130.

FITZGERALD, G. H., 1933, Gavano : a new specific for amœbic dysentery. *Indian med. Gaz.*, **LXVIII.**, 458.

HALAWANI, A., 1930, Experimental study on the resistance of *Entamœba histolytica* to emetine hydrochloride *in vitro*. *Ann. trop. Med.*, **XXIV.**, 273.

KNOWLES, R., NAPIER, L. E., and DAS GUPTA, B. M., 1923, A preliminary note on the relationship of the intestinal protozoa of man to the hydrogen ion concentration of their environment. *Indian med. Gaz.*, **LVIII.**, 151.

MANSON-BAHR, P., 1931, Modern methods of treatment of intestinal amœbiasis. *Proc. R. Soc. Med.*, **XXIV.**, 1538.

Martindale, W. H., 1923[1], Solubility of emetine-bismuth iodide. *Lancet*, *i.*, 158.
Martindale, W. H., 1923[2], A note on emetine preparations for rectal and oral use. *Trans. R. Soc. trop. Med. Hyg.*, XVII., 27.
Reed, A. C., 1930 (quoted by Anderson, H. H., and Leake, C. D., 1930).
St. John, J. H., 1933, The effect of emetine on *Endamœba histolytica* in culture. *Amer. J. Hyg.*, XVIII., 414.

THE ACTION OF EMETINE AND OTHER ALKALOIDS ON ENTAMŒBA HISTOLYTICA

The discovery of a fluid medium, in which amœbæ would grow freely, enabled the action of drugs on *Entamœba histolytica* to be readily studied. The original findings of Laidlaw, Dobell and Bishop (1928) that emetine hydrochloride would inhibit the growth of *E. histolytica* in a dilution of 1 in 5,000,000 have been amply confirmed by St. John (1933) and Nossina (1934), both of whom have emphasised the important *rôle* played by physical factors such as *p*H, increased acidity decreasing the activity of the drug. The dilution in which emetine acts on amœbæ suggests that *in vivo* also the action of emetine is direct.

Under the same conditions as emetine the *in vitro* action of the other ipecacuanha alkaloids on cultures of *E. histolytica* is not very marked :

Alkaloid.	Minimum concentration inhibiting growth and the reaction of the medium.		
	*p*H		
Emetine hydrochloride . . .	7·0		1 in 5,000,000
Cephaeline hydrochloride . .	6·8		1 in 1,000,000
*iso*Emetine hydrochloride . .	6·8		1 in 10,000
Emetamine hydrochloride . .	7·0		1 in 5,000
Demethoxyemetine hydrochloride .	7·0	>	1 in 5,000
Psychotrine sulphate . . .	7·0	>	1 in 5,000
N-methylpsychotrine sulphate .	6·8	>	1 in 5,000
Norcephaeline hydrochloride. .	6·8	>	1 in 5,000

An amœbicidal action is also developed *in vitro* by harmine hydrochloride, which at *p*H 7 inhibits the growth of *E. histolytica* in a dilution of 1 in 20,000, while harmaline hydrochloride is inhibitory in a dilution of 1 in 50,000. Coulthard (1934) has tested the *in vitro* action of a number of harmol and alkylharmol derivatives. The *in vivo* action of these alkaloids has not been tested.

Little action *in vitro* is exhibited by the cinchona alkaloids, but benzylquitenine hydrochloride was active in a dilution of 1 in 100,000, the quitenine series showing an increase in amœbicidal action with increase in molecular weight up to the benzyl compound.

Alkaloid.	Minimum concentration lethal to *E. histolytica*.
Hydrochloride of:	
Harmol . . .	1 in 40,000 to 1 in 80,000
o-Ethylharmol . .	1 in 80,000
o-*n*-Propylharmol .	1 in 80,000 to 1 in 120,000
o-*n*-Butylharmol. .	1 in 20,000 to 1 in 80,000
o-*n*-Hexylharmol .	1 in 100,000 to 1 in 200,000
o-*n*-Heptylharmol .	1 in 200,000
o-*n*-Octylharmol .	1 in 200,000 to 1 in 300,000
o-*n*-Nonylharmol .	1 in 200,000 to 1 in 500,000
o-*n*-Decylharmol. .	1 in 100,000
o-*n*-Dodecylharmol .	> 1 in 100,000
o-*iso*Propylharmol .	> 1 in 80,000
o-*sec*-Octylharmol .	> 1 in 200,000
o-Allylharmol . .	1 in 80,000
o-Benzylharmol . .	1 in 80,000

o-*n*-Nonylharmol is, however, sparingly soluble in water, and in order to obtain more soluble compounds the corresponding dialkylamino derivatives were compared with the amœbicidal action of emetine. Although the most active member of the series was found by Pyman (1937) to be less amœbicidal than emetine, it possessed several times the activity of *o*-*n*-Nonylharmol. It was, in fact, found that amœbicidal action was due not so much to the harmol residue, but to the dialkylaminoalkyl group. A series of tetra-alkyldiamino paraffins were therefore studied, and of these $\alpha\kappa$-tetra-*n*-amyldiaminodecane was found not only to have a greater amœbicidal action *in vitro* than emetine, but to be one-third to one-eleventh as toxic for mice as emetine. Unfortunately, this compound proved too irritant for parenteral administration in man and not sufficiently active to be of value when given orally.

References

COULTHARD, C. E., 1934, The chemotherapy of derivatives of harmine and harmaline, II. *Biochem. J.*, XXVIII., 264.

LAIDLAW, P. P., DOBELL, C., and BISHOP, A., 1928, Further experiments on the action of emetine in cultures of *Entamœba histolytica*. *Parasitology*, XX., 207.

NOSSINA, V., 1934, Action of drugs upon *Entamœba histolytica in vitro*. *Med. Parasitol. Moscow*, III., 451.

PYMAN, F. L., 1937, Researches in chemotherapy. (Presidential Address, Section B (Chem.), Brit. Ass., Nottingham.) *Chemistry and Industry*, LXI., 789.

ST. JOHN, J. H., 1933, The effect of emetine on *Endamœba histolytica* in culture. *Amer. J. Hyg.*, XVIII., 414.

QUINOLINE DERIVATIVES

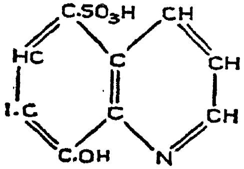
Since emetine is an *iso*quinoline derivative, other compounds of this group have been tested for amœbicidal action. Child and Pyman (1929) prepared seven derivatives consisting of two *iso*quinoline rings connected by a single carbon chain where the *iso*quinoline rings were substituted in the same way as in emetine. The amœbicidal action of these compounds has not been tested *in vivo*, but *in vitro* they were found to have no inhibitory effect on *Entamœba histolytica* in dilutions lower than 1 in 5,000.

More extensive investigations on the halogenated oxyquinolines have resulted in the discovery of two compounds which have both been used in the treatment of amœbic dysentery.

IODOHYDROXYQUINOLINESULPHONIC ACID

Iodohydroxyquinolinesulphonic acid (*chiniofon* B.P. Addendum, *yatren, loretin, quinoxyl, mixiod, anayodin*) is 7-iodo-8-hydroxyquinoline-5-sulphonic acid mixed with approximately

20 per cent. of sodium bicarbonate. It contains about 28 per cent. of iodine and its structural formula is said to be :



It is a pale yellow powder which easily absorbs moisture and must therefore be kept as dry as possible. At ordinary temperatures it is soluble in water to the extent of 4 per cent. For some years under the name of loretin the drug was used as an intestinal disinfectant, but in 1921 Mühlens and Menk reported its use in the cure of eight resistant cases of amœbic dysentery. Since then it has been extensively employed in the treatment of both acute and chronic amœbiasis. It may be administered either by mouth or *per rectum* in the form of an enema containing 2·5 per cent. of the drug. By mouth it is best given in tablet form, each tablet containing 0·25 gm. of the drug. While chiniofon has often been given alone, it is now more often administered in combination with either emetine and bismuth iodide or acetarsol. When chiniofon is given with emetine and bismuth iodide it is not necessary to restrict the diet to the same extent as when the emetine compound is given alone. Manson-Bahr (1931) noted only two relapses among 181 patients treated with chiniofon *per rectum* and emetine and bismuth iodide by mouth. In children Panayotatou (1929) prefers to combine chiniofon enemata with acetarsol by mouth.

Although chiniofon generally rapidly clears up the diarrhœa, and in most cases eliminates amœbæ from the intestine, it should be remembered that it is quite useless in the treatment of amœbic hepatitis and amœbic abscess, nor is it of use against other intestinal protozoa. In a few patients chiniofon may increase diarrhœa and cause a scalding sensation in the rectum during defæcation. A reduction in the daily dose of the drug is usually sufficient to remove these symptoms.

Toxic symptoms are, as a rule, rare, but Wolff (1936) mentions the case of a young girl whose urine became loaded with urobilin after she had taken only six chiniofon pills. Cases of acute and subacute yellow atrophy with fatty degeneration of the heart and kidneys have been recorded after intravenous inoculation (*cf.* Maxon, 1933). In rabbits, according to Dyckerhoff (1935), intravenous injections produce liver necrosis and degeneration of the kidney tubules, the hepatic lesions being specially marked when the liver glycogen is first depleted. Kessel (1928) found that monkeys receiving 2 gm. of chiniofon daily died with evidence of liver damage. Chiniofon should not therefore be injected intravenously, and care should be taken to watch for early symptoms of liver insufficiency. For reasons which are as yet unknown, compounds containing the quinoline ring appear specially liable to produce necrosis of the liver parenchyma.

IODOCHLOROHYDROXYQUINOLINE

In a study of the effects of the halogenation of oxyquinoline on biological activity, Anderson, David and Koch (1931) found that with increased halogenation of oxyquinoline and in proportion to the atomic weight of the halogen there was increased toxicity and a comparable increase in balanticidal action, but no increased amœbicidal activity *in vitro*. Of eleven compounds investigated the most promising was iodochlorohydroxyquinoline (vioform N.N.R.). Vioform was originally prepared as a substitute for iodoform. It contains about 40 per cent. iodine, in comparison with 28 per cent. in yatren, and 12 per cent. chlorine. A greyish-yellow powder, with a faint aromatic odour, it is soluble in glacial acetic acid, but almost insoluble in water and only sparingly soluble in alcohol. The hydrochloride, which is soluble in water, is irritating to mucous membranes in dilutions of 1 in 500, while emulsions of the insoluble compound also irritate the lower bowel. Vioform cannot therefore be given per rectum. A comparison of the biological activity of iodochlorohydroxyquinoline (vioform) and iodohydroxyquinolinesulphonic acid (chiniofon) has been made by David, Johnstone, Reed and Leake (1933), who give the following particulars :

Biological activity.	Vioform.	Chiniofon.
Toxicity for guinea-pigs on single oral administration.	200 mgm./kgm. kills 13/20 guinea-pigs in 4 days.	900 mgm./kgm. kills 7/15 guinea-pigs in 6
In vitro amœbicidal concentration in 24 hours.	Sol. hydrochloride 1 : 10,000.	1 : 500.
Balanticidal activity in naturally infected guinea-pigs.	"Cured" 80 per cent. at divided total dosage of 150 mgm./kgm. with 20 per cent. mortality.	"Cured" 60 per cent. at divided total dosage of 600 mgm./kgm. with 40 per cent. mortality.
Amœbicidal activity in naturally infected rhesus monkeys (Anderson and Koch, 1931).	"Cured" 7/8 at 100 mgm./kgm. for 10 days.	"Cured" 2 at 500 mgm. daily for 28 days: killed 2 at 2 gm. daily for 10 days.

Vioform was first tested for amœbicidal activity on macacus monkeys naturally infected with *E. histolytica.* The results were excellent, all the treated animals gained in weight and there was no evidence of toxicity. In rabbits, killed after the administration of 100 mgm. per kilogram of body weight daily for ten days, no gross or microscopic lesions were found, but with a single dose of 250 mgm. per kilogram of body weight, given by mouth, there was damage to the renal tubules and in the liver fatty infiltration and scattered foci of necrosis. As in the case of chiniofon, careful watch should therefore be kept for evidence of liver insufficiency. David and his co-workers (1933) gave 0·25 gm. in capsules three times a day for ten days. After a week's rest the course could be repeated. The stools became an oily green colour. As a rule there are no unpleasant reactions, though occasionally patients complain of flatulence, colic and diarrhœa. To avoid these symptoms the drug may be given in coated pills. A considerable number of patients have now been treated with vioform, and in the great majority the amœbæ have disappeared after a single course of treatment. David and his colleagues found that of forty-four cases followed for from one to three months thirty-eight remained cured. Similar results have been obtained in Egypt by Azmy and Taha (1934), who gave 1 gm. a day for ten days, and El Biblawi (1936), who found that pregnancy was no contraindication, in Italy by Giordano (1934), who obtained a "cure" in twenty-eight out of thirty cases, in Japan by Akashi (1934), who gave a course of 0·75 gm. daily for fifteen days.

Neither vioform nor chiniofon has any very marked effect on *E. histolytica in vitro.* Their curative action *in vivo* may therefore possibly be bactericidal rather than directly amœbicidal and due to the killing off of certain of the bacteria on which the amœbæ live.

DIIODOHYDROXYQUINOLINE

Diiodohydroxyquinoline ("diodoquin"), $C_9H_4NI_2OH$, is said to contain 63·9 per cent. of iodine. It is extremely insoluble in water, dilute acids and alkalies, and only slightly soluble in the common organic solvents. Dogs which received 4 to 6 gm. orally a day for six days failed to develop toxic symptoms. Most of the drug is recovered in the excretions, the iodine being still united with the quinoline nucleus. Tenney (1936) claims to have used this compound with success in the treatment of human amœbiasis, but no clinical comparison with the effects of yatren and vioform has yet been made.

References

AKASHI, K., 1934, The treatment of amœbiasis with iodochlorhydroxyquinoline. *J. med. Ass. Formosa,* XXXIII., 1801 [English summary, p. 156].

ANDERSON, H. H., DAVID, N. A., and KOCH, D. A., 1931, Effects of the halogenation of oxyquinoline on biological activity. *Proc. Soc. exp. Biol. N. Y.*, XXVIII., 484.

ANDERSON, H. H., and KOCH, D. A., 1931, Iodochloroxyquinoline (Vioform N.N.R.) as an amebacide in macaques. *Proc. Soc. exp. Biol. N.Y.*, XXVIII., 838.

AZMY, S., and TAHA, S., 1934, The treatment of amœbiasis by iodochlorhydroxyl-quinoline. *J. Egypt. med. Ass.*, XVII., 809.

CHILD, R., and PYMAN, F. L., 1929, Bases containing two *iso*quinoline rings. *J. chem. Soc.*, Pt. II., 2010.

DAVID, N. A., JOHNSTONE, H. G., REED, A. C., and LEAKE, C. D., 1933, The treatment of amebiasis with iodochlorhydroxyquinoline (Vioform N.N.R.). *J. Amer. med. Ass.*, C., 1658.

DYCKERHOFF, H., 1935, Ueber Leberschädigung durch Yatren. *Münch. med. Wschr.*, LXXXII., 1802.

El Biblawi, A., 1936, The treatment of the different forms of dysentery and other intestinal disorders. *J. Egypt. med. Ass.*, XIX., 39.

Giordano, M., 1934, Contributo alla terapia dell'amebiase intestinale. *Arch. ital. Sci. med. colon.*, XV., 706.

Kessel, J. F., 1928, Intestinal protozoa of monkeys. *Univ. Calif. Publ. Zool.*, XXXI., 275.

Manson-Bahr, P., 1931, Modern methods of treatment of intestinal amœbiasis. *Proc. R. Soc. Med.*, XXIV., 1538.

Maxon, H., 1933, Zwei Fälle von Leberschädigung nach Yatrenbehandlung. *Zbl. Chir.*, LX., 879.

Mühlens, P., and Menk, W., 1921, Ueber Behandlungsversuche der chronischen Amœbenruhr mit Yatren. *Münch. med. Wschr.*, LXVIII., 802.

Panayotatou, A., 1929, Ueber einige Fälle chronischer Amöben-Enterocolitis bei Kindern. *Arch. Schiffs- u. Tropenhyg.*, XXXIII., 595.

Tenney, A. C., 1936, The present concept of *Entameba histolytica* infestation. *Illinois med. J.*, LXX., 145.

Wolff, E., 1936, Zur Frage der Leberschädigung durch Yatren. *Münch. med. Wschr.*, LXXXII., 1966.

KURCHI

The bark and seed of *Holarrhena antidysenterica* Stapf., a small deciduous tree belonging to the Apocynaceæ and found throughout India and Burma, have long been valued as a remedy for dysentery in the form of an infusion of kurchi, the Bengali name for the plant. An alkaloid, conessine ($C_{12}H_{20}N$), was obtained by Haines (1858, 1865) from the bark: when injected subcutaneously it causes necrosis, but it can be given orally or intravenously without causing any local reaction. When given intravenously it causes a prolonged and well-marked fall of blood pressure. In large doses, according to Bakhsh (1936^{2}), it slows and weakens the action of the heart: *iso*conessine is approximately half as toxic. In addition to the alkaloid conessine, Pyman (1919) isolated holarrhenine from kurchi, while Ghosh and Ghosh (1928) obtained two other akaloids—kurchine and kurchicine—from the bark (*cf.* Ghosh and Bose, 1932). More recently, Haworth (1932) has isolated a base norconessine ($C_{23}H_{28}N_2$) which appears to be identical with kurchine. The physiological properties of norconessine have been investigated by White (1933) and are very similar to those of conessine, though its amœbicidal action *in vitro* is less, for whereas conessine prevented the growth of *E. histolytica* in a buffered medium at *p*H 7·2 in a dilution of 1 : 20,000, norconessine was inhibitory only in 1 : 5,000. Dioxyconessine was also active only in the same concentration. Yet another base, lettocine ($C_{17}H_{25}O_2N$), has been isolated from kurchi bark by Peacock and Chowdhury (1935). The pharmacology of kurchicine has been studied by Chopra, Gupta and Chopra (1933), who find that it acts as a general protoplasmic poison, and in toxic doses causes slowing of the heart and eventually complete heart block. Intravenous injection causes a fall of blood pressure. Similar results have been obtained by Bakhsh (1936^1). Decoctions of kurchi bark have long been used in India as a popular folk remedy, but modern interest in the drug dates from the work of Brown (1922) and Henry and Brown (1923), who demonstrated its lethal effect on free-living protozoa. The mixed alkaloids of kurchi are much less toxic than emetine and appear to be without depressant, irritant or emetic effects. Accounts of its amœbicidal action in man vary considerably. Leake (1932) and others in America failed to demonstrate any curative action ; Acton and Chopra (1933), on the other hand, found that intramuscular injections of 2 gr. of conessine hydrochloride produced as rapid a cure as emetine, but were very painful. Kurchi bismuth iodide, however, given in doses of 10 gr. twice daily half an hour after a mixture containing 40 gr. of sodium citrate and 1 drachm of sodium bicarbonate, produced excellent results in seventy-eight cases, the proportion of cures to failures being 3·16 : 1, while with emetine and bismuth iodide the ratio was 1 : 3·5. Ghosh (1933) believes that the curative action of kurchi bark depends not only on the alkaloidal content, but also on the tannins, mucilage and resinous matter. An extract of the bark made with 60 per cent. alcohol contains all this material, but in a 90 per cent. alcoholic extract there is some loss of water-soluble substances.

References

Acton, H. W., and Chopra, R. N., 1933, The treatment of chronic intestinal amœbiasis with the alkaloids of *Holarrhena antidysenterica* (Kurchi). *Indian med. Gaz.*, LXVIII., 6.

Bakhsh, I., 1936^1, Actions of Kurchicine, an alkaloid of *Holarrhena antidysenterica*. *J. Pharmacol.*, LVIII., 361.

Bakhsh, I., 1936^2, The pharmacological actions of conessine and *iso*conessine. *Ibid.*, LVIII., 373.

Brown, H. C., 1922, Observations on the amœbicidal action of conessine. *Brit. Med. J.*, *i.*, 993.

Chopra, R. N., Gupta, J. C., and Chopra, G. S., 1933, Pharmacological action of Kurchicine (an alkaloid of *Holarrhena antidysenterica*). *Indian J. med. Res.*, XXI., 277.

Ghosh, A. B., 1933, A study on the preparation of an efficient extract of Kurchi (*Holarrhena antidysenterica*). *Indian med. Gaz.*, LXVIII., 13.

Ghosh, S., and Bose, I. B., 1932, Die Alkaloide der Rinde von *Holarrhena antidysenterica* (Kurchi Bark). II. Teil. *Arch. Pharm. Berl.*, CCLXX., 100.

Ghosh, S., and Ghosh, N. N., 1928, The alkaloids of Kurchi bark (*Holarrhena antidysenterica*). Part I. A preliminary note on two new alkaloids discovered in Indian Holarrhena. *J. Indian chem. Soc.*, V., 477.

Haines, R., 1858, Nereine: a new vegetable alkaloid extracted from the bark of the *Wrightia antidysenterica. Trans. med. phys. Soc. Bombay*, IV., Appen., p. xxviii.

Haines, R., 1865, Notes on conessine *alias* wrightine. *Pharm. J.*, VI., 432.

Haworth, R. D., 1932, A new Alkaloid from *Holarrhena antidysenterica* seeds. *J. chem. Soc.*, 631.

Henry, T. A., and Brown, H. C., 1923, The influence of the medium on the toxicity of certain alkaloids towards protozoa. *Trans. R. Soc. trop. Med. Hyg.*, XVII., 61.

Leake, C. D., 1932, Chemotherapy of amebiasis. *J. Amer. med. Ass.*, XCVIII., 195.

Peacock, D., and Chowdhury, J. C., 1935, A new alkaloid from the bark of *Holarrhena antidysenterica. J. chem. Soc.*, 734.

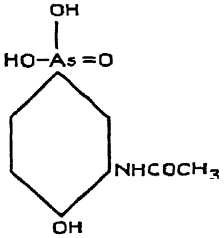
Pyman, F. L., 1919, Alkaloids of *Holarrhena congolensis* Stapf. *J. chem. Soc.*, CXV., 163.

White, A. C., 1933, The physiological action of norconessine. *J. Pharmacol.*, XLVIII., 79.

ORGANIC ARSENICAL COMPOUNDS

(A) Acetylaminohydroxyphenylarsonic Acid

3-Acetylamino-4-hydroxyphenylarsonic acid (*acetarsol*, B.P. Addendum, 1936, *stovarsol*, *spirocid*, *kharophen*, *goyl*, *acetphenarsine*, *osarsol*, *osvarsan*, *stovarsolan*, *orarsan*, *acetarsone* N.N.R.), which has the following graphic formula :



Acetarsol.

is a white powder practically insoluble in water and with an arsenic content of 27·2 per cent. It was originally studied by Ehrlich and Hata (1911) and re-investigated by Fourneau and his colleagues (1921 and 1923). It has been used in the treatment of syphilis, yaws and malaria, as well as in amœbiasis. Marchoux (1923) appears to have been the first to employ it in the treatment of chronic cases of amœbic dysentery. According to Newman and Davies (1926), who treated alternate cases with emetine hydrochloride injections and acetarsol by mouth, the clinical symptoms disappeared in from four to five days with acetarsol as against eight days with emetine; a third of the patients treated with acetarsol relapsed, however, as against only 11 per cent. of those treated with emetine.

Leake (1932) found that monkeys naturally infected with *Entamœba histolytica* tolerated a total dose of 400 mgm. per kilogram of body weight given in daily doses of 30 mgm. per kilogram of body weight with but slight effect on the parasitic amœbæ. *In vivo* amœbæ were killed only in a concentration of 1 in 600.

Although acetarsol, in common with other quinquevalent arsenicals, produces in the majority of human beings an increased sense of well-being, it may also give rise to very pronounced toxic symptoms and even death. Bender (1927) has reported malaise, fever, œdema, jaundice, diarrhœa, albuminuria, coryza, bronchitis and skin disorders such as diffuse erythema, dryness and pruritus. Brown (1935) treated 232 cases of amœbiasis without a death, but thirteen persons suffered from toxic erythemata, in some cases so severe as to amount to exfoliative dermatitis. One patient had serious peripheral neuritis which did not clear up for nearly a year. Anderson and Reed (1934[1]) state that one out of every six patients shows some intolerance and gastric distress.

Acetarsol, after oral administration, is in part excreted in the urine. In one normal human subject, Chen, Anderson and Leake (1930) found that 7 per cent. of the arsenic ingested in 0·5 gm. of acetarsol was found in the urine after twenty-four hours and 20 per cent. after seventy-two hours.

The majority of workers now prefer to combine acetarsol with emetine. The combination of acetarsol with emetine, however, greatly reduces amœbicidal activity, for whereas emetine *in vitro* kills *E. histolytica* in a dilution of 1 in 5,000,000, emetine acetarsol kills only in a dilution of 1 in 100,000. Acetarsol has also been given in conjunction with chiniofon enamata in the treatment of amœbiasis in children.

(B) Formylaminohydroxyphenylarsonic Acid

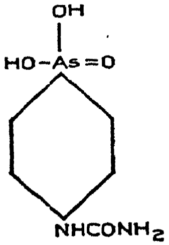
3-Formylamino-4-hydroxyphenylarsonic acid (tréparsol), containing 28·75 per cent. of arsenic, was first employed by Flandin (1924), who gave 0·25 gm. twice daily. Tréparsol is more rapidly excreted in the urine than is acetarsol and thus possibly has a rather larger margin of safety (Brown and Osterberg, 1931). Brown (1935) treated 301 cases of amœbic dysentery; eight showed toxic erythema, four at the end of the first course of 3 to 3·75 gm. and four during or at the end of the second course of 3 gm. The symptoms were not severe and subsided in from three to five days.

Brown believes that the secret of success in the treatment of amœbiasis lies in the employment of a variety of drugs. Thus if no treatment has recently been given 0·065 gm. of emetine hydrochloride is first given subcutaneously twice daily for three days. After an interval of a week 0·043 gm. of emetine is given twice daily for three or more days. With the institution of the emetine, tréparsol 0·25 gm. is administered orally with each meal for four days. If there is no intolerance to arsenic, two more such courses are prescribed with intervals of ten days between the courses.

If stool tests are positive following this regimen three courses of chiniofon are prescribed, and if these fail arsphenamine and bismuth subnitrate are administered. Very few cases of infection persist after this varied treatment.

(C) Carbamidophenylarsonic Acid

4-Carbamidophenylarsonic acid (*carbarsone*, N.N.R., *amibiarson*), which contains 28·85 per cent. of arsenic when anhydrous, was first prepared by Ehrlich and Bertheim (1909). It is a white crystalline solid, stable in air and practically insoluble in water, though it dissolves readily in alkaline aqueous solutions and melts at 174° C.



Carbarsone.

On oral administration it is absorbed from the intestine, and according to Chen, Anderson and Leake (1930) is excreted in the urine, the rate of excretion being very slightly slower than in the case of acetarsol. When injected parenterally, however, carbarsone is first excreted into the small intestine, chiefly in the bile; from the bowel it is re-absorbed to be again excreted by the kidney. After single minimal lethal doses, administered in gelatine capsules by mouth (150 mgm. per kilogram of body weight in guinea-pigs, 200 mgm. per kilogram in rabbits, from 200 to 250 mgm. per kilogram of body weight in cats), animals show symptoms of lethargy, loss of weight, abdominal distension, polyuria, diarrhœa, tremor, sluggish reflexes, loss of balance, incoordination and failure of the pupils to respond to light. Subpericardial hæmorrhage is not uncommon in rats.

Rats, according to Gabaldon (1936[1, 2]), are more resistant, for the M.L.D. by mouth is in excess of 6,500 mgm. per kilogram of body weight. Old rats are more susceptible than young. Histological examination of animals dying after such toxic doses shows evidence of necrosis of the renal tubules. According to Hogue (1934) carbarsone *in vitro* has very little action on the cells of the intestinal tract of eight-day-old chick embryos.

On repeated oral administration of doses within the therapeutic range (50 mgm. per kilogram of body weight daily for ten days in monkeys) no toxic symptoms were noted, and in rabbits killed by air injection, after receiving 30 mgm. per kilogram of body weight daily for ten days, no evidence of tissue injury was found on microscopical examination.

Carbarsone is thus less toxic to animals than stovarsol. On the other hand, David, Anderson, Koch and Leake (1931) found that carbarsone *in vitro* killed amœbæ in a dilution of 1 in 4,000, while under similar conditions stovarsol was lethal only in a dilution of 1 in 600. The chemotherapeutic index of carbarsone is stated to be 1 : 8·8.

Reed, Anderson, David and Leake (1932) state that of 175 persons with amœbiasis only four continued to show the presence of amœbæ in the stools after a single course of treatment. This treatment, which caused no toxic reactions, consisted of 75 mgm. per kilogram of body weight in divided doses spread over a period of at least ten days, since after oral administration the arsenic in the compound is rather slowly absorbed and eliminated. In the case of the average adult this means that 0·25 gm. is given by mouth twice daily for ten days.

The largest dose which caused no symptoms of arsenical poisoning was 204 mgm. per kilogram of body weight given over a period of six weeks ; the smallest dose which proved effective in destroying amœbæ was 29 mgm. per kilogram of body weight. Anderson and Reed (1934^2), in a further series of 330 cases treated with carbarsone, found no evidence of damage to the skin, optic nerves or kidneys, though some of the patients experienced slight gastric upsets, and one, with a history of previous liver damage, developed an acute hepatitis, which rapidly disappeared within five days of stopping the drug.

Chopra, Sen and Sen (1933), and Chopra and Sen (1934), in a series of fifty-three cases treated for ten or fifteen days with 0·25 gm. twice daily by mouth, found that the stools, as a rule, became free from amœbæ three to four days after the beginning of treatment. The proportion of probable cures to failures was 1 : 0·12 ; no toxic reactions were noted, while the general tonic effect common to arsenicals was very pronounced.

A complete absence of toxic reactions has also been noted in patients given from 150 to 1,200 mgm. per kilogram of body weight over a period of forty-eight weeks. A number of mild reactions have, however, been reported in human beings, ranging from nausea, vomiting, diarrhœa, headache, sore throat, lachrymation and sneezing to such severe symptoms as generalised pruritis and exfoliative dermatitis, laryngeal and pulmonary œdema, retinal œdema and papillitis, granular casts and albumin in the urine, and icterus. Certain cases of dysentery fail to react either to carbarsone or to any other amœbicidal drugs.

The only fatal case so far recorded is that described by Epstein (1936) in a woman who, for the treatment of giardiasis, took 5 gm. of carbarsone (83·3 mgm. per kilogram of body weight) by mouth over a period of ten days. She became disorientated, diarrhœa continued, the skin was erythematous and covered with a scaly eruption: jaundice supervened, the liver enlarged and death occurred fifteen days after admission to hospital. At autopsy the findings were those of an acute yellow atrophy of the liver, with some degeneration of the convoluted tubules of the kidney.

It is thus obvious that carbarsone, though it may produce fewer reactions than acetarsol, is by no means free from danger, while from its close chemical similarity to tryparsamide it is not improbable that its prolonged use may give rise to symptoms of optic atrophy.

Chopra, Sen and Sen (1935) report on the use of a compound termed "Amibiarson," which is said to have a chemical constitution very similar to that of carbarsone and, like it, is given orally in doses of 0·25 gm., in gelatine capsules, twice a day for from ten to fifteen consecutive days. The patients remain on an ordinary diet and a saline purge is taken with the drug.

Of forty patients who received this treatment twenty-five were cured: in ten the result was indeterminate and, in five the treatment failed. The ratio of probable cures to failures was, therefore, 5 : 1; with carbarsone it was 5·7 : 1. Anderson and Reed (1934 [1]) claim that carbarsone is very effective if given *per rectum* as a 1 per cent. solution in 200 c.c. of 2 per cent. sodium bicarbonate solution.

The parasiticidal action of carbarsone is apparently not restricted to *Entamœba histolytica*, for Gabaldon (1935 and 1936 [2]) has found that *Trichomonas hominis* and rat trichomonads are readily destroyed, the action *in vivo* and *in vitro* being closely parallel.

(D) OTHER ARSENICAL PREPARATIONS

From time to time claims have been made that arsphenamine and neoarsphenamine have a specific action in the treatment of amœbiasis. Any beneficial results that have been obtained seem to be due to the tonic effects of the arsenic rather than to a specific action on the amœbæ. David and his co-workers (1931) found that with increase in the number of carbon atoms in the side-chain of arsenical quinquevalent compounds of the carbarsone type an increase in toxicity occurred, the pathological changes being similar in type to those produced by stovarsol. Sodium *N*-phenyl-β-aminoproprionamide-*p*-arsonate—"proparsamide," $NH_2COCH_2.CH_2.NH.C_6H_4.AsO_3HNa$, containing 26 per cent. arsenic, was tolerated by monkeys in daily doses of 50 mgm. per kilogram of body weight up to a total of 800 mgm. per kilogram of body weight. Amœbæ disappeared only during the actual administration of the drug.

The substitution of an atom of sulphur for oxygen in the carbamido radical of *p*-carbamidophenylarsonic acid with the production of $AsO_3H_2C_6H_4.NHCSNH_2$ produces, according to Leake, Koch and Anderson (1930), a considerable reduction in toxicity: amœbicidal activity, however, is likewise decreased. Compounds such as arsenic trithiosalicylic acid were found by Leake (1932) to have little effect on eradicating *Entamœba histolytica* from monkeys except in doses which were lethal.

References

ANDERSON, H. H., and LEAKE, C. D., 1930, Toxicity of acetarsone. *Proc. Soc. exp. Biol. N.Y.*, XXVII., 267.

ANDERSON, H. C., and REED, A. C., 1934 [1], Carbarsone rectally in amebiasis. *Amer. J. trop. Med.*, XIV., 257.

ANDERSON, H. C., and REED, A. C., 1934 [2], Untoward effects of anti-amebic drugs. *Amer. J. trop. Med.*, XIV., 269.

BENDER, W. L., 1927, Stovarsol (spirocid) poisoning: report of six cases. *Amer. J. med. Sci.*, CLXXIV., 819.

BROWN, P. W., 1935, Results and dangers in the treatment of amœbiasis. A summary of fifteen years' clinical experience at the Mayo Clinic. *J. Amer. med. Ass.*, CV., 1319.

BROWN, P. W., and OSTERBERG, A. E., 1931, Toxicity and rate of elimination of organic arsenicals (stovarsol and treparsol) in treatment of endamebiasis. *Amer. J. med. Sci.*, CLXXXII., 257.

CHEN, M.-Y., ANDERSON, H. H., and LEAKE, C. D., 1930, Rate of urinary arsenic excretion after giving acetarsone ("stovarsol") and "carbarsone" by mouth. *Proc. Soc. exp. Biol. N.Y.*, XXVIII., 145.

CHOPRA, R. N., and SEN, S., 1934, Carbarsone in intestinal amœbiasis. Part II. *Indian med. Gaz.*, LXIX, 375.

CHOPRA, R. N., SEN, B., and SEN, G., 1933, Treatment of chronic intestinal amœbiasis with carbarsone. *Indian med. Gaz.*, LXVIII., 315.

CHOPRA, R. N., SEN, B., and SEN, G., 1935, Amibiarson in the treatment of chronic intestinal amœbiasis. *Indian med. Gaz.*, LXX., 324.

DAVID, N. A., ANDERSON, H. H., KOCH, D. A., and LEAKE, C. D., 1931, Comparative toxicity and protozoacidal action of acetarsone, carbarsone and certain related pentavalent arsenical compounds. *Proc. Soc. exp. Biol. N.Y.*, XXIX., 125.

EHRLICH, P., and BERTHEIM, A., 1909, U.S. Patent 937929.

EHRLICH, P., and HATA, S., 1911. "Die experimentelle Chemotherapie der Spirillosen." Berlin.

EPSTEIN, E., 1936, Toxicity of carbarsone. Acute fatty degeneration of the liver, exfoliative dermatitis and death following its administration. *J. Amer. med. Ass.*, CVI., 769.

FLANDIN, C., 1924, Le dérivé formylé de l'acide méta-amino-para-oxy-phényl-arsinique (tréparsol) dans le traitement de l'amibiase. *Bull. Soc. méd. Hôp. Paris.*, XLVIII., 1628.

FOURNEAU, E., 1921, Sur l'emploi de l'acide oxyaminophénylarsinique et des acides arylarsiniques en général dans le traitement des spirilloses et des trypanosomiases. *Ann. Inst. Pasteur*, XXXV., 571.

FOURNEAU, E., NAVARRO-MARTIN, A., and TRÉFOUËL, M. and Mme., 1923, Les dérivés de l'acide phénylarsinique (arsenic pentavalent) dans le traitement des trypanosomiases et des spirilloses expérimentales. *Ann. Inst. Pasteur*, XXXVII., 551.

GABALDON, A., 1935, Carbarsone : its action on *Trichomonas hominis* and on rat trichomonads *in vitro*. *Amer. J. Hyg.*, XXII., 326.

GABALDON, A., 1936[1], Pharmacological observations on 4-carbamino-phenyl-arsonic acid (carbarsone) in rats. *Amer. J. Hyg.*, XXIII., 122.

GABALDON, A., 1936[2], Carbarsone : its action on the intestinal trichomonads of rats *in vivo*. *Amer. J. trop. Med.*, XVI., 621.

HOGUE, M. J., 1934, Further studies on the effect of amœbicidal drugs on tissue culture cells (arsenious trithiosalicylic acid, carbarsone, Kurchi bismuth iodide, proparsamide, vioform). *Amer. J. trop. Med.*, XIV., 443.

LEAKE, C. D., 1932, Chemotherapy of amœbiasis. *J. Amer. med. Ass.*, XCVIII., 195.

LEAKE, C. D., KOCH, D. A., and ANDERSON, H. H., 1930, Effect on biological activity of substituting sulphur for oxygen in an organic arsenical compound. *Proc. Soc. exp. Biol. N.Y.*, XXVII., 717.

MARCHOUX, E., 1923, Le stovarsol guérit rapidement la dysentérie amibienne. *Bull. Soc. Path. exot.*, XVI., 79.

NEWMAN, R. E. U., and DAVIES, R. M., 1926, A comparison of stovarsol and emetine in the treatment of amœbic dysentery. *J. R. Army med. Cps.*, XLVII., 296.

Reed, A. C., Anderson, H. H., David, N. A., and Leake, C. D., 1932, Carbarsone in the treatment of amebiasis. *J. Amer. med. Ass.*, **XCVIII.**, 189.
Reed, A. C., and Johnstone, H. G., 1934, Amebiasis among one thousand prisoners. *Amer. J. trop. Med.*, **XIV.**, 181.

OTHER AMŒBICIDAL DRUGS

(i.) **Rivanol,** which is 2-ethoxy-6 : 9-diaminoacridine lactate, was originally prepared by Morgenroth as an antiseptic and has been used in the treatment of bacillary dysentery. It was recommended as an amœbicide by Peter (1927) and Wagner (1928), but Biggam and Arafa (1930) have failed to show that it has any curative action.

(ii.) **Bismuth subnitrate** was originally introduced about 1908 by Deeks in the treatment of amœbic dysentery in Panama, the results being analysed in a paper published in 1914. James and Deeks (1925) subsequently advocated combined treatment with bismuth salts and emetine. According to Acton and Knowles (1924), repeated doses of bismuth carbonate tend to increase the alkalinity of the gut content and thus favour the amœbicidal action of emetine.

No critical examination of the amœbicidal action of bismuth salts has yet been made, but it should not be forgotten that methæmoglobinæmia has been recorded as a result of the administration of bismuth subnitrate (Roe, 1933).

(iii.) **Iodaseptine Cortial,** a French patent primarily designed for the treatment of chronic rheumatism and tuberculosis, is described as " iodobenzomethyformine." Africa and Garcia (1935) have used it in the treatment of five chronic cases of amœbic dysentery. The symptoms of chronic diarrhœa rapidly and permanently disappeared, and in one instance cysts had not returned three years after the end of treatment.

(iv.) **Iodoform.** In subacute and chronic cases of amœbic colitis the use of iodoform has been recommended by Castellani (1935). Treatment necessitates the patient remaining in bed on a very light or fluid diet, and after a purge of magnesium sulphate iodoform is given in keratinized capsules each containing 0·05 gm., one or two capsules being given three to four times daily for twelve or fifteen days. After an interval of a week the course may be repeated. The drug may also be given by enema, 0·2–0·3 gm. in 300 c.c. water. In two cases amœbæ disappeared from the stools on the fourth and sixth days after treatment was started, while among seven other patients the only complaint was of slight giddiness twelve days after the beginning of treatment.

(v.) **Hexylresorcinol.** Faust (1930) has suggested the use of hexylresorcinol, but others have failed to show that it has an amœbicidal action.

References

ACTON, H. W., and KNOWLES, R., 1924, On the dysenteries of India. *Indian med. Gaz.*, LIX., 325.

AFRICA, C. M., and GARCIA, E. U., 1935, Iodaseptine Cortial (Iodobenzomethyformine) in the treatment of chronic amœbiasis. *J. P. I. med. Ass.*, XV., 305.

BIGGAM, A. G., and ARAFA, M. A., 1930, The effect of rivanol in the treatment of amœbic dysentery. *Lancet*, *i.*, 1335.

CASTELLANI, A., 1935, The treatment of amœbic colitis with iodoform. *J. trop. Med.* (*Hyg.*), XXXVIII., 268.

DEEKS, W. E., 1914, Dysenteries in the Canal Zone, with special reference to amœbic dysentery. *Ann. trop. Med. Parasit.*, VIII., 321.

FAUST, E. C., 1930, Effect of di-hydranol on intestinal protozoa and laboratory animals. *Proc. Soc. exp. Biol. N.Y.*, XXVII., 905.

JAMES, W. M., and DEEKS, W. E., 1925, The etiology, symptomatology and treatment of intestinal amebiasis. *Amer. J. trop. Med.*, V., 97.

PETER, F. M., 1927, Rivanol in der Behandlung der Amöbendysenterie. *Münch. med. Wschr.*, LXXIV., 1709.

ROE, H. E., 1933, Methemoglobinemia following the administration of bismuth subnitrate. Report of a fatal case. *J. Amer. med. Ass.*, CI., 352.

WAGNER, O., 1928, Untersuchungen über die experimentelle Chemotherapie der Amöbenruhr. *Beih. Arch. Schiffs- u. Tropenhyg.*, XXXII., 159.

CHAPTER III

THE CHEMOTHERAPY OF LEISHMANIASIS

THE use of antimony in the treatment of visceral leishmaniasis constitutes one of the most important chemotherapeutic advances of the present century, for before the introduction of antimony the death rate from kala-azar in India was almost 90 per cent. The reason why antimony, alone among the elements, should have a specific action on *Leishmania donovani*, but not to the same extent, if at all, on *L. tropica*, is as yet unexplained. Two periods may be distinguished in the treatment of leishmanial infections, an earlier period in which antimonyl compounds of the tartar emetic type were exclusively used, and a later one characterized by the employment of quinquevalent antimony derivatives.

The chemotherapy of antimony compounds has recently been fully discussed by Schmidt and Peter (1938).

KALA-AZAR

The credit for bringing to the notice of the medical world the specific action of antimony in leishmanial infections is due to Gaspar Vianna (1912), who used tartar emetic with success in the treatment of South American leishmaniasis. Confirmation of his results was quickly forthcoming. In Italy Di Cristina and Caronia (1915) found the drug highly efficient in the treatment of infantile kala-azar, while Rogers (1915) used it with success in India, to be quickly followed by Muir (1915), Mackie (1915) and others.

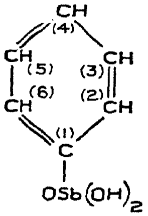
As toxic effects occasionally followed the use of potassium antimonyl tartrate, Rogers (1918) gave up its use in favour of sodium antimonyl tartrate. This preparation also must of necessity be given intravenously. Like other antimonyl tartrates, it not infrequently predisposes to broncho-pneumonia. Antimonyl tartrates have now therefore been largely discarded in favour of the less toxic quinquevalent preparations, though in Italy Caronia (1930) still remains faithful to their use, especially where the cost of treatment must be carefully considered. In small children also the administration of antimonyl tartrates *per rectum* has been suggested as a means of avoiding the necessity of a series of hypodermic injections. Panayotatou (1929) claims to have cured a child in Alexandria with rectal injections of tartar emetic, a total of 0·05 gm. being given. In India, Saha (1931) has used the same method of administration. Sulphurated antimony containing antimony sulphides, antimony oxides and free sulphur has also been recommended by Tournier (1931). It has an advantage in that it can be given by mouth, either in cachets or in a draught, in doses up to 0·4 gm. three to four times daily, without causing vomiting or other unpleasant symptoms. Its use has not been sufficiently extensive to determine whether it is equal or even superior to tartar emetic in efficiency.

" Antimosan " (p. 47) was tested by Mühlens (1926) and Struthers (1927), but its curative action is very slow.

" Neoantimosan," or " fouadin " (p. 47), proved ineffective in the hands of Wigmore and Cameron (1931) : antimony in the tervalent form, in fact, is almost without action on *Leishmania donovani*.

QUINQUEVALENT ANTIMONY COMPOUNDS

In the organic compounds of antimony, however, there have been discovered substances which fulfil the requirements of reduced toxicity to the host and increased toxicity to the parasite. The preparation of quinquevalent antimonial drugs was first made practicable in 1911, following the discovery of a method of synthesis by Schmidt (1920). In this process, by the interaction



Phenylstibonic Acid

of diazotized aniline and antimony trioxide in the presence of alkali, there is formed phenylstibonic acid.

An examination of the structural formula of phenylstibonic acid shows that the therapeutically active element, antimony, is directly united to a carbon atom, while in the emetic series it is joined to carbon through oxygen.

A large number of compounds can be formed by substitution of one or more hydrogen atoms in positions 2 to 6 in the benzene nucleus by atoms, such as Cl, or groups, such as the amino or hydroxyl. In all these organic antimony compounds the antimony is not ionized in solution and therefore does not give the usual analytical reactions of " inorganic antimony," until the associated organic residue has been destroyed.

The quinquevalent antimony compounds so far prepared can be classed under three headings :—

(I) Salts of *para*-aminophenylstibonic acid :
  (*a*) Sodium *para*-aminophenylstibonate—" stibamine."
  (*b*) Diethylamine *para*-aminophenylstibonate—" von Hevden 693," " von Heyden 693B," " neostibosan."
  (*c*) Urea salt of stibanilic acid—" urea-stibol."
(II) Derivatives obtained by substitution in the amino-group of *para*-aminophenylstibonic acid :
  (*a*) Sodium *para*-acetylaminophenylstibonate—" stibacetin," " stibenyl."
  (*b*) Urea and glucose combined with *para*-aminophenylstibonate—" amino-stiburea."
  (*c*) Nitrogen glucoside of sodium *para*-aminophenylstibonate —" neostam."
  (*d*) " Urea stibamine "—active component the di-substituted urea, *s*-diphenylcarbamide—4 : 4′-distibonic acid.
  (*e*) Sodium *para*-*N*-phenylglycineamide-*p*-stibonate.
(III) Derivatives obtained by substitution in the benzene nucleus of *para*-aminophenylstibonic or *para*-acetylaminophenylstibonic acid :
  Sodium *meta* - chloro-*para* - acetylaminophenylstibonate— " stibosan "—" von Heyden 471."

The first of these compounds to be tested was sodium *para*-

aminophenylstibonate ($NH_2.C_6H_4.SbO(OH)(ONa)$) (*Stibamine*), which is the antimony analogue of atoxyl. This compound, when examined by Uhlenhuth, Mulzer and Hügel (1913), and later by Brahmachari (1922), proved too unstable in solution and too uncertain in its action to be used therapeutically.

Sodium *para*-acetylaminophenylstibonate (" *stibacetin*," later known as " *stibenyl* ") ($CH_3.CO.NH.C_6H_4SbO(OH)(ONa)$) was, however, much more stable and less toxic, for Fargher and Gray (1921) found that the M.L.D. for mice was 133 mgm. per kilogram of body weight, compared with the minimal lethal doses for sodium and potassium antimonyl tartrate of 25 mgm. and 16 mgm. respectively. In fowl spirochætosis and rabbit syphilis also "stibenyl" was found to have a more favourable chemotherapeutic index than tartar emetic.

In the meantime, Brahmachari (1922) had introduced a product which he termed " urea-stibamine." This substance was at first stated to be urea combined with *para*-aminophenylstibonic acid, with the empirical formula $C_7H_{12}O_4N_3Sb$. Later it was suggested that the compound was ammonium-*para*-carbamidophenylstibonate, but the work of Ghosh and his colleagues (1928) and Gray *et al.* (1931) has shown that, as available in commerce, " urea-stibamine " is not a single substance. The latter workers have shown that, in addition to a di-substituted urea, *s*-diphenylcarbamide-4 : 4′-distibonic acid, it contains also antimonic acid, together with some *p*-acetylaminophenylstibonic acid, resulting from the incomplete hydrolysis of the stibacetin used as a starting substance ; owing to the weakly acid character of these materials, they retain only a small amount of ammonia, which suffices, in consequence of their predominantly colloidal nature, to bring the whole into solution. The antimony content of various commercial samples was shown by Ghosh, Chopra and Chatterjee (1928) to vary between 20 and 43 per cent. Napier (1927) found that the lethal dose for mice was 250 mgm. per kilogram of body weight, while the tolerated dose was 175 mgm. A compound known as " novostiburea " has been used by Sur and Neogi (1933) for the treatment of kala-azar in India. It is stated to be the sodium salt of " urea-stibamine," but in view of what has been said in regard to the composition of " urea-stibamine " this statement must be accepted with reserve.

Two other compounds somewhat on the same lines as Brahmachari's " urea-stibamine " have been introduced, " aminostiburea " and " urea-stibol." The former is said to be the ammonium salt of *para*-aminophenylstibonic acid combined with urea and glucose. The toxic dose for mice is given by Napier (1927) as 250 mgm. per kilogram of body weight, the tolerated dose as 175 mgm. " Urea-stibol " was introduced by Chopra, Gupta, Mullick and Gupta (1928). No formula has been provided, but it is claimed that the drug is a salt of urea and *para*-aminophenylstibonic acid. It is said to be toxic for mice on intravenous injection of from 220 to 230 mgm. per kilogram of body weight.

Another derivative of *sodium* para-*aminophenylstibonate* has been prepared by Gray (1925), who has produced a stable *N*-glucoside. It is used therapeutically under the name " neostam." Napier (1927) gives the lethal dose for mice as 500 mgm. per kilogram of body weight, while the tolerated dose is 300 mgm.

Another quinquevalent antimony derivative which requires notice is " stibosan " or " von Heyden 471." This compound is formed by the substitution of a chlorine atom in the benzene nucleus of *para*-acetylaminophenylstibonic acid with the formation of sodium *meta*-chloro-*para*-acetylaminophenylstibonate ($(CH_3.CO.NH)(Cl)C_6H_3.SbO(OH)(ONa)$). The lethal dose for mice was found by Napier (1927) to be 275 mgm. per kilogram of body weight, the tolerated dose 200 mgm.

Finally, " von Heyden 693," or " neostibosan," must be described. This compound, diethylamine *para*-aminophenylstibonate, was found by Napier (1927) to be lethal for mice in doses of 350 mgm. per kilogram of body weight. At first, when used clinically, it was liable to produce vomiting in certain patients. By a slightly different method of preparation this drawback was at least partially eliminated and the toxic properties of the compound greatly reduced.

One disadvantage of " neostibosan " is that when dissolved in water it does not make a very stable solution. This drawback has been overcome by a new quinquevalent antimony compound, " solustibosan " No. 561, the composition of which is not yet disclosed, although according to Schmidt (1937) it is a derivative of a hexonic acid containing quinquevalent antimony. It is a clear colourless solution which curiously enough does not give the serum precipitin test; 1 c.c. contains 20 mgm. of metallic antimony, thus corresponding closely in metallic content with 1 c.c. of a 5 per cent. solution of " neostibosan," which contains 21 mgm. of antimony. It is said to be less toxic than " neostibosan "; 18·5 c.c. per kilogram of body weight is the lethal dose for a mouse, as against 6 c.c. of a 5 per cent. " neostibosan " solution. Its rate of excretion in the urine in man is rapid, 68·5 per cent. being excreted in the first twenty-four hours, instead of 50 per cent. in the case of " neostibosan." According to Weese (1937) mice survive the subcutaneous injection of 43 c.c. or 860 mgm. of Sb per kilogram of body weight, that is to say, nearly 5 per cent. of the total body weight, while " neostibosan " kills 50 per cent. of mice when 615 mgm. of Sb is given. In the rabbit 15 to 18·5 c.c., 300 to 370 mgm. Sb per kilogram of body weight, leads to transient collapse. Intravenous injections up to 1·5 c.c. per kilogram of body weight caused no pathological changes in the liver and kidneys of rabbits. Kikuth and Schmidt (1937) found that in experimental leishmaniasis of the European hamster, *Cricetus frumentarius*, the amount of metallic antimony necessary to bring about a cure with " solustibosan " was somewhat larger than in the case of " neostibosan ":—

	" Neostibosan."	" Solustibosan."
Effective dose . .	200 mgm. = 84 mgm. Sb.	6·5 c.c. = 130 mgm. Sb.
Slightly effective dose .	100 ,, = 42 ,, ,,	4 ,, = 80 ,, ,,
Ineffective dose . .	50 ,, = 21 ,, ,,	2 ,, = 40 ,, ,,

In addition to these compounds, Brahmachari (1933) has used a compound described as sodium sulphomethylstibanilate which is said to contain 24·5 per cent. of antimony. Rats can stand up to 0·4 gm. per kilogram of body weight, given intravenously, without showing toxic symptoms, while doses of 0·5 gm. have been given to human beings by intramuscular injections without causing local or constitutional disturbance. Brahmachari, Das Gupta, Banerjea and Basu (1930) have employed a compound described as sodium-*N*-phenylglycineamide-4-stibonate, the antimony analogue of tryparsamide. " Stibional," an Italian preparation of undisclosed composition, but stated to contain 0·018 gm. of antimony in 1 c.c., has given good results in the hands of Lereboullet, Chabrun and Baize (1931), while Raybaud (1935) has suggested that the antimony compounds " Dn 7 " and " Dn 18," which appear to be antimonyl derivatives of hydroxyquinolinesulphonic acids, might be of value in the treatment of visceral leishmaniasis.

The use of either the striped Chinese hamster (*Cricetulus griseus*, M. Edw.) or preferably the European hamster (*Cricetus frumentarius*), which is highly susceptible to leishmanial infection, provides a reliable method of determining the chemotherapeutic index of antimony preparations. Roehl (1929), for instance, has found that with this animal the chemotherapeutic index of " stibosan " is 1 : 5 to 1 : 7, that of " neostibosan " 1 : 50.

THE CLINICAL APPLICATIONS OF QUINQUEVALENT ANTIMONY COMPOUNDS

The first of the quinquevalent antimony compounds to be tested clinically was " stibenyl," which was employed in Italy by Caronia (1916) in the treatment of cases of kala-azar. In the Mediterranean area the use of " stibenyl " has continued (Caronia, 1930 ; Lignos, 1932), since it can be given intramuscularly or even subcutaneously. Lignos, for instance, gave a total of thirty injections of from 6 to 12 cgm. at three-day intervals and cured 31 out of 33 cases of infantile leishmaniasis. In India, on the other hand, " stibenyl " proved quite ineffective. Toxic symptoms were extremely common, possibly owing to the fact that the compound was unsuitable for export to the tropics.

Up to the present no careful comparison of " neostam," " neostibosan " and " urea-stibamine " has been made under strictly similar conditions, so that there is little published evidence available bearing on the relative efficiencies of these three drugs, which all continue to be used in India and China. As Lee and Chu (1935) point out, " urea-stibamine " is somewhat more potent than " neostibosan," for the full course for an adult of the former is from 1·5 to 2·5 gm. and of the latter 4 to 5 gm. On the other hand, " urea-stibamine " has no definite chemical composition and may be more toxic than " neostibosan." Brahmachari and Brahmachari (1931), however, have treated 125 patients by an intensive method involving the intravenous injection of " urea-stibamine " either daily or even several times a day for a period of six to nine days, the total daily dose varying from 0·025 to 0·2 gm., and claim to have obtained cures with a minimum of toxic reaction.

Struthers (1931), in China, and Napier (1932 [1 & 2]), in India, have both reported favourably on the use of " neostibosan." Struthers treated 87 cases, beginning with an initial adult intravenous dose of 0·1 gm. daily or every other day, and rising to 0·2 or 0·3 gm., though the last amount in daily doses was not as well tolerated as in India. Total adult doses amounted to from 2·6 to 3 gm. In children injections were given either intravenously or intramuscularly, the initial dose being 0·05 gm., followed by 0·2 gm. on alternate days. Of fifty patients who had completed treatment, twenty-eight of them six to nine months previously, three only had died, while forty-seven were in good health.

In India, Napier (1932 [2]) had up to the end of 1928 treated 254 patients : of these, six died during treatment and four failed to respond to treatment, while of 217 persons who were traced six months after cessation of treatment five died within six months of discharge from some other disease and thirteen relapsed. Thus, if the failure rate, the death rate during treatment and the relapse rate are taken into account, the cure rate is 90·08 per cent. As all the cases that failed to respond to treatment had been previously treated with other antimony preparations and had relapsed, it follows that the cure rate of primarily treated patients is even better than this. Actual total doses of 2·25 gm. or relative doses of 3 gm. per kilogram of body weight were found most suitable, the best course being five injections on five consecutive days. " Neostam " had been used in India by Napier (1929), who reported on the treatment of 57 cases. Of forty-four patients whose subsequent history could be traced, thirty-five remained in good health while nine relapsed. Of sixteen who received a relative total dose of more than 3 gm. (mean 4·32 gm.) only one relapsed. The drug was well tolerated in comparatively large doses. Yates (1929) and Low (1929) have also obtained favourable results from "neostam." In India 10 cases of kala-azar have been treated by Napier, Chaudhuri and Rai Chaudhuri (1937) with solustibosan, the immediate results being very satisfactory. In China solustibosan has been employed by Struthers and Lin (1937) and Yates (1937). Struthers and Lin treated twenty-nine patients, of whom three died during treatment, three failed to complete the course, one failed to respond to treatment and twenty-two were discharged as cured. Yates (1937) obtained cures in 78 of 82 cases. Five daily doses of solustibosan were well tolerated and no reactions were noted. The drug may be given subcutaneously, intravenously or intramuscularly. More prolonged observations are required to determine whether the cure is permanent. Napier (1932[2]) is of opinion that, contrary to the generally held belief, early cases do not respond to treatment as well as those with a long history of fever, provided that these patients have not previously been subjected to antimony treatment. Although on occasions patients may fail to react to one antimony compound, it must not be assumed that the parasites have therefore become antimony resistant, or rather resistant to the benzene ring, in the sense that trypanosomes become resistant. Not infrequently, as in the treatment of amœbic dysentery, a change from one antimony preparation to another will ensure rapid improvement.

THE CRITERIA OF CURE

The best evidence of cure by the quinquevalent antimony compounds is an entire absence of clinical symptoms for a period of six months after the termination of treatment. Relapses after this period are extremely rare. From the size of the spleen and the weight of the patient at the time of discharge little or no information of any prognostic value can be obtained, but according to Napier (1932 [2]), when the total leucocyte count was done one week after treatment, no case with a count of over 6,000 per c.mm. relapsed, though only 25 per cent. of the cases below this figure did so. The formol leucogel (aldehyde) reaction may also be of value, for it becomes negative when serological cure has occurred. It is not improbable, however, that the degree of reaction occurring in the aldehyde test is an indication of the degree of immunity occurring in the patient. Nevertheless, Chopra and Chaudhury (1932) believe that if the time of gelation in the aldehyde test begins to increase about three weeks after the course of injections has been completed the probability is that the patient is showing a good response to treatment. Prognosis may also be indicated by watching the progress of the " protein graph," as described by Lloyd, Napier and Paul (1929), but at present there are no clinical or laboratory methods that will do more than indicate the probability of successful cure by organic compounds of antimony.

THE ADVANTAGES OF THE QUINQUEVALENT AS COMPARED WITH THE TERVALENT ANTIMONY COMPOUNDS

The advantages which are claimed for the quinquevalent antimony compounds as compared with the tervalent derivatives, that is to say, the emetic type of antimonial drug, are as follows :

The toxicity of the quinquevalent compounds is much less, they can therefore be administered in larger doses. The total amount of antimony necessary to effect a cure can be administered in a much smaller number of doses. Ten to twelve doses of quinquevalent compounds, as against thirty doses of sodium antimonyl tartrate, can be looked on as the average number of injections necessary for an ordinary case. This reduction in number means that the time required for treatment is reduced from two months to three weeks, or in the case of " neostibosan " to eight or ten days. The necessity for intravenous injections is avoided. Resistant cases which show little or no improvement with the tartrates rapidly improve when given relatively large doses of quinquevalent antimony compounds. On the other hand, certain cases which fail to respond to quinquevalent compounds have been cured by injections of tartrates.

Disagreeable symptoms, such as cough and severe joint pains frequently associated with the tartrate treatment, as well as the liability to broncho-pneumonia, are less likely to occur when quinquevalent compounds are used. The death rate amongst kala-azar patients has been very markedly reduced since the introduction of quinquevalent preparations. Thus Zahra-Neumann (1933) gives the following figures for infantile kala-azar treated in Malta. In a first series of 100 cases treated with tartar emetic the death rate was 26·5 per cent.; with greater experience in the administration of the drug the death rate among the next 124 cases was 17·7 per cent., but among 68 cases treated with " neostibosan " it was reduced to 10·17 per cent. Very similar results have been recorded from India, for Napier (1926), in a series of 167 cases treated during 1925 with six different quinquevalent antimony compounds, had a death rate of only 4·2 per cent.

The disadvantages of the quinquevalent compounds are few. Jaundice is perhaps rather more common in patients treated with these compounds, though according to Guha (1935) the liver function was normal in 23 cases after treatment with " urea-stibamine." With some of the quinquevalent compounds, such as " aminostiburea " and " urea-stibamine," an anaphylactic-like condition is by no means uncommon : with " stibosan " it is rare and with " neostibosan " it has not been seen. The symptoms, according to Chopra (1927), are associated with extreme changes in the pulmonary blood pressure, together with contraction of the bronchial musculature. Unless very large doses are given intravenously the symptoms rapidly disappear.

METHOD OF ACTION OF ANTIMONY COMPOUNDS IN KALA-AZAR

The means by which antimony destroys the parasites of leishmania in the body is at present unknown. *In vitro*, as

Noguchi (1924) has shown, tartar emetic has very little action on cultures of leishmania. *In vivo* also the action is almost certainly not a direct one. Acton and Chopra (1927) believe that the parasiticidal action of antimony is dependent on a number of factors. The parasites are destroyed by antimony not in the skin, but in the reticulo-endothelial cells of the spleen, liver and bone-marrow. This suggests that antimony may act by stimulating the reticulo-endothelial cells to the production of parasiticidal substances.

The effect of antimony on the bone-marrow is to increase the number of leucocytes which helps to digest the parasites not actually within endothelial cells. Rhythmic contraction of the spleen may help to release parasites from heavily infected cells, while increased functioning of the adrenals dilates the vessels of the liver and spleen, thereby increasing capillary permeability and diminishing the nutrition of the endothelial cells. The question has been further studied by Boyd, Napier and Roy (1931), who injected sublethal doses of " neostibosan " and found that no particular organ or system has any marked affinity for antimony. The immediate distribution is dependent entirely on physical factors, the main channel of elimination being through the kidneys. Forty-eight hours after the last dose of a course, the bulk of the antimony is found in the liver, where it is possibly stored before excretion. There is, however, no general loading of the reticulo-endothelial cells of the body with antimony. These observations do not lend support to the theory that antimony acts by stimulation of the reticulo-endothelial system, though they cannot be said to disprove it, since stimulation need not be accompanied by storage of the stimulating substance : they do, however, support the contention that the action of antimony on the leishmanial parasites is an indirect one. Napier and Krishnan (1931) suggest that an important factor in producing a cure is the immunity response of the individual patient.

Further investigations must obviously be undertaken before the curative action of the quinquevalent antimony compounds can be fully understood.

References

Acton, H. W., and Chopra, R. N., 1927, The action of the pentavalent compounds on the *Leishmania donovani* parasites. *Trans. 7th Congr. Far-East. Ass. trop. Med., British India,* III., 1.

Boyd, T. C., Napier, L. E., and Roy, A. C., 1931, The distribution of antimony in the body organs. *Indian J. med. Res.,* XIX., 285.

Brahmachari, P., and Brahmachari, U., 1931, The intensive treatment of kala-azar. *J. trop. Med. (Hyg.),* XXXIV., 263.

Brahmachari, U. N., 1922, Chemotherapy of antimonial compounds in kala-azar infection. Part I. *Indian J. med. Res.,* X., 492.

Brahmachari, U. N., 1933, Studies in kala-azar and chemotherapy of antimony. Part VI. Treatment of kala-azar with intramuscular injection of sodium sulphomethyl stibanilate. *Trans. R. Soc. trop. Med. Hyg.,* XXVI., 389.

Brahmachari, U., Das Gupta, J. M., Banerjea, R., and Basu, B., 1930, Studies in kala-azar and chemotherapy of antimony. Part II. The treatment of kala-azar with intramuscular injection of sodium-*n*-phenylglycineamide-4-stibinate. *Trans. R. Soc. trop. Med. Hyg.,* XXIII., 617.

Caronia, G., 1916, L'impiego di nuovi preparati di antimonio per via intramuscolare nella cura della leishmaniosi infantile. *Pediatria,* XXIV., 65.

Caronia, G., 1930, The therapy of internal leishmaniasis. *Amer. J. trop. Med.,* X., 261.

Chopra, R. N., 1927, An experimental investigation into the action of organic compounds of antimony. *Indian J. med. Res.,* XV., 41.

Chopra, R. N., and Chaudhury, S. G., 1932, The prognostic value of the aldehyde reaction in kala-azar. *Indian med. Gaz.,* LXVII., 260.

Chopra, R. N., Gupta, J. C., Mullick, M. N., and Gupta, A. K. D., 1928, Urea stibol in the treatment of kala-azar. *Indian med. Gaz.,* LXIII., 252.

Di Cristina, G., and Caronia, G., 1915, Sulla terapia della leishmaniosi interna. *Pediatria,* XXIII., 81.

Fargher, R. G., and Gray, W. H., 1921, The chemotherapy of antimony. Comparison of the antimonyl tartrates with the organic compounds of antimony. *J. Pharmacol.,* XVIII., 341.

Ghosh, S., Chopra, R. N., and Chatterjee, N. R., 1928, Urea stibamine: its preparation and composition. *Indian J. med. Res.,* XVI., 461.

Gray, W. H., Trevan, J. W., Bainbridge, H. W., and Attwood, A. P., 1931, The ureides of *p*-aminophenylstibinic acid. *Proc. Roy. Soc. B.,* CVIII., 54.

Gray, W. H., and The Wellcome Foundation Ltd. 1925, E. P. 234,677.

Guha, P. R., 1935, Studies in kala-azar. With special reference to the liver function before and after treatment with pentavalent antimony compounds (urea stibamine). *Calcutta med. J.,* XXX., 193.

Kikuth, W., and Schmidt, H., 1937, Contribution to the progress of antimony therapy of kala-azar. *Chin. med. J.,* LII., 425.

Lee, C. U., and Chu, C. F., 1935, Relative value of urea stibamine and neostibosan in the treatment of kala-azar. *Chin. med. J.,* XLIX., 328.

Lereboullet, P., Chabrun, J., and Baize, P., 1931, Un cas de kala-azar infantile observé à Paris. *Bull. Soc. méd. Hôp. Paris,* XLVII., 932.

Lignos, A., 1932, Note sur le traitement du kala-azar infantile. *Bull. Soc. Path. exot.,* XXV., 1043.

Lloyd, R. B., Napier, L. E., and Paul, S. N., 1929, The serological control of treatment of kala-azar, with observations on the significance of hypoproteinæmia. *Indian J. med. Res.,* XVI., 1065.

LOW, G. C., 1929, An interesting case of kala-azar from the point of view of diagnosis. *Trans. R. Soc. trop. Med. Hyg.*, **XXIII.**, 305.

MACKIE, P., 1915, Tartar emetic in kala-azar. *Brit. med. J.*, *ii.*, 745.

MÜHLENS, P., 1926, Ein mit Antimosan geheilter Fall von indischem Kala-azar. *Arch. Schiffs- u. Tropenhyg.*, **XXX.**, 160.

MUIR, E., 1915, The treatment of kala-azar with tartar emetic. *Indian med. Gaz.*, L., 365.

NAPIER, L. E., 1926, The pentavalent compounds of antimony in the treatment of kala-azar. I. Stibosan. *Indian J. med. Res.*, **XIV.**, 263.

NAPIER, L. E., 1927, "Kala-azar." 2nd edit., London.

NAPIER, L. E., 1929, The pentavalent compounds of antimony in the treatment of kala-azar. Part V. Stibamine glucoside (neostam). An analysis of the treatment in fifty-seven consecutive cases. *Indian J. med. Res.*, **XVI.**, 911.

NAPIER, L. E., 1932[1], The pentavalent compounds of antimony in the treatment of kala-azar. Part VI. A comparison of results with different compounds. *Indian J. med. Res.*, **XIX.**, 705.

NAPIER, L. E., 1932[2], The pentavalent compounds of antimony in the treatment of kala-azar. Part VII. Neostibosan: di-ethyl-amine *para*-aminophenyl stibiate: 254 cases. *Indian J. med. Res.*, **XIX.**, 719.

NAPIER, L. E., CHAUDHURI, R. N., and RAI CHAUDHURI, M. N., 1937, A stable solution of antimony for the treatment of kala-azar. *Indian med. Gaz.*, **LXXII.**, 462.

NAPIER, L. E., and KRISHNAN, K. V., 1931, A theory of the ætiology and epidemiology of kala-azar in India. *Indian med. Gaz.*, **LXVI.**, 603.

NOGUCHI, H., 1924, Action of certain biological chemical and physical agents upon cultures of *Leishmania:* some observations on plant and insect herpetomonads. *Proc. int. Conf. Hlth. Problems trop. Amer.*, p. 455.

PANAYOTATOU, A., 1929, Infantile kala-azar: a case cured at Alexandria by a new method. *Brit. J. Child. Dis.*, **XXVI.**, 112.

RAYBAUD, A., sen., 1935, Quelques nouveaux sels d'antimoine. *Marseille méd.*, **LXXII.**, 465.

ROEHL, W., 1929, Chemotherapeutic investigations with antimony preparations in the experimental kala-azar of the hamster. *Indian med. Gaz.*, **LXIV.**, 563.

ROGERS, L., 1915, Tartar emetic in kala-azar. *Brit. med. J.*, *ii.*, 197.

ROGERS, L., 1918, Sodium antimonyl tartrate vel tartar emetic in kala-azar. *Indian med. Gaz.*, **LIII.**, 161.

SAHA, B., 1931, Treatment of kala-azar by 25 per cent. aqueous solution of sodium antimony tartrate and résumé of other non-intravenous methods. *Calcutta med. J.*, **XXV.**, 335.

SCHMIDT, H., 1920, Über aromatische Antimonverbindungen. *Liebigs Ann.*, **CDXXI.**, 174.

SCHMIDT, H., 1937, Die Entwicklung der Kala-azar Mittel. *Festschrift Prof. Nocht.*

SCHMIDT, H., and PETER, F. M., 1938, "Advances in the therapeutics of antimony." Leipzig.

STRUTHERS, E. B., 1927, The treatment of kala-azar by stibosan (Heyden 471) and antimosan (Heyden 661). *Chin. med. J.*, **XLI.**, 755.

STRUTHERS, E. B., 1931, Neostibosan in the treatment of kala-azar. Notes on the treatment of 87 cases. *Chin. med. J.*, **XXVIII.**, 1.

STRUTHERS, E. B., and LIN, L. C., 1937, The treatment of kala-azar with solustibosan, a new antimony compound. *Chin. med. J.*, **LII.**, 335.

SUR, S. N., and NEOGI, S. K., 1933, "Novostiburea" in the treatment of kala-azar. *Indian med. Gaz.*, **LXVIII.**, 273.

TOURNIER, 1931, Le kermès ; ses applications en pathologie exotique. *Ann. méd. Pharm. colon.*, XXIX., 871.

UHLENHUTH, P., MULZER, P., and HÜGEL, G., 1913, Die chemotherapeutische Wirkung von organischen Antimonpräparaten bei Spirochäten- u. Trypanosomenkrankheiten. *Dtsch. med. Wschr.*, XXXIX., 393.

VIANNA, G., 1912, Sociedade Brasileira de Dermatologia, 4a Sessão Ordinaria. *Arch. brasil. Med.*, II., 422.

WEESE, H., 1937, Important pharmaceutical aspects of solustibosan. *Chin. med. J.*, LII., 421.

WIGMORE, J. B. A., and CAMERON, W. M., 1931, Two cases of infantile kala-azar. *J. R. Army med. Cps.*, LVI., 449.

YATES, T. M., 1929, Treatment of kala-azar, with report of 92 cases from Hope Hospital, Anhwei, China. *Chin. med. J.*, XLIII., 1053.

YATES, T. M., 1937, " Sdt. 561 " in the treatment of kala-azar. *Chin. med. J.*, LII., 339.

ZAHRA-NEUMANN, C., 1933, A report on the treatment of infantile kala-azar. *Trans. R. Soc. trop. Med. Hyg.*, XXVI., 383.

CUTANEOUS AND MUCO-CUTANEOUS LEISHMANIASIS

Under this heading are included (i.) oriental sore, due to *Leishmania tropica* ; (ii.) the muco-cutaneous form of leishmaniasis, espundia, found in South America, and the very similar condition occasionally met with in parts of Africa, such as the Anglo-Egyptian Sudan ; and (iii.) the dermal leishmaniasis first described in India by Brahmachari (1922) and most frequently associated with visceral infections.

For the last condition Napier (1927) has found that the only treatment is the intravenous injection of one or other of the quinquevalent antimony compounds. Those cases of dermal leishmaniasis that are associated with a previous visceral infection are sometimes very refractory to antimony, and one patient received over 5 gm. of stibosan with little or no improvement. A series of twenty-three patients treated by Napier and Haldar (1930) were cured, however, by intravenous injections of "aminostiburea." An average total dose of 5 gm. was given in an average period of 123·1 days, the average number of injections being 26·7. Brahmachari and Banerjea (1931) gave six intravenous injections of " urea-stibamine " associated with local inunctions of metallic antimony.

ORIENTAL SORE

After the introduction of tartar emetic in South American leishmaniasis by Gaspar Vianna (1912), antimonyl tartrates were also employed in the treatment of oriental sore. The records of their use are not numerous, and intravenous injection of tartar emetic has been regarded by many as a somewhat drastic procedure for a disease usually without serious consequences; many other remedies have since been recommended. The difficulty of determining the value of any particular drug in the treatment of oriental sore lies in the fact that the disease tends to spontaneous healing. Dostrowsky (1929), for instance, found that spontaneous cure might occur after from three to seventeen months.

Intramuscular injections of tervalent antimony compounds such as "antimosan" have proved of little value, and the same is true of quinquevalent preparations, though Joannidès (1931) claims to have obtained a cure in twenty-two days by combining local treatment with intramuscular injections of "neostibosan," while Kousa (1935) combines intravenous injections of "fouadin" with local applications of a 25 per cent. solution of iron perchloride. The response to antimony injections is nearly always slow, but in most cases the sores do eventually respond. When the sores are heavily infected with other organisms local treatment is essential.

Among other drugs that have been used are iodobismuthate of quinine (Vigne and Fournier, 1928), emetine hydrochloride (Photinos, 1930), an arsenic-antimony preparation of undisclosed composition "Sdt. 386B" (Sincke, 1935) and various gold preparations such as "krysolgan" and "sanocrysin" (Sorinson, 1932) or potassium gold cyanide and "solganol" (Krasnjanskij and Lawrow, 1936). Laqueur (1932) recommends an ointment made of equal parts of camphor, olive oil and beeswax, though this is contraindicated in sores near the nose and eyelids as it sometimes leads to extension of the ulcerative process. In anæmic patients tonics containing arsenic and strychnine aid the healing process (Laqueur, 1933).

Few critical attempts have been made to determine the exact value of any of these drugs; more intensive experiments have been carried out with berberine acid sulphate ("orisol") (*cf.* Chopra *et al.*, 1932).

The alkaloid berberine, isolated in 1826, occurs in many species of *Berberis*, and is usually extracted from barberry bark (*Berberis vulgaris*) or obtained as a by-product in the manufacture of hydrastine from the Golden Seal, *Hydrastis canadensis*. A crude extract made from berberis plants has long been used in Indian indigenous medicine under the name of "rasaut," and as long ago as 1911 Jolly used "rasaut" in the treatment of oriental sore. The alkaloid berberine was first used by Varma (1927), and later by Karamchandani (1927) and others. Gupta (1930), who used a 2 per cent. solution of the acid sulphate, injected 3 c.c. at a time and points out the importance of infiltrating both the sore and surrounding hyperæmic area. Hayward (1933) found that all cases that received five or more injections were cured, but the injections caused so much pain that many patients refused to continue treatment. Chatterjee (1934) gave 1 c.c. injected through two punctures for each sore, the injections being given every other day till twelve infiltrations had been made. Das Gupta and Dikshit (1929) claim that berberine sulphate has a direct action *in vitro* on both *Leishmania tropica* and *L. donovani*. Pützer (1934), however, finds that only those berberine derivatives which form soluble salts with acids show any effect on the lesions. Warma (1934) prefers to combine injections of a 1 per cent. solution with a vaccine made from cultures of the causative organism.

MUCO-CUTANEOUS LEISHMANIASIS

If the treatment of oriental sore is still uncertain, that of muco-cutaneous leishmaniasis is even more unsatisfactory, since, for reasons which are at present unknown, the muco-cutaneous lesions of South American leishmaniasis are much more resistant to antimony than infections due to *Leishmania tropica*. This is especially true of the mucous lesions, which yield to antimony far less readily than those of the skin.

The quinquevalent antimony compounds have proved valueless, but Mazza and Arias Aranda (1931) treated two cases in the Argentine with intramuscular injections of "fouadin." The first two injections consisted of 1·5 and 3·5 c.c., subsequent injections 5 c.c. In both cases rapid healing of the lesions took place: one patient received thirty-two injections, the other seventeen in just over two months. More recently Elias (1936) claims to have successfully treated three other patients with South American leishmaniasis by intramuscular injections of " fouadin." The curious oral leishmaniasis occurring in the Sudan responds to intravenous injections of tartar emetic or " neostibosan " (Humphreys and Mayne, 1935).

References

Brahmachari, U. N., 1922, A new form of cutaneous leishmaniasis. *Indian med. Gaz.*, **LVII.**, 125.

Brahmachari, U., and Banerjea, R., 1931, Studies in kala-azar and chemotherapy of antimony. Part V. The treatment of resistant cases of dermal leishmanoid. *Trans. Roy. Soc. trop. Med. Hyg.*, **XXIV.**, 565.

Chatterjee, R., 1934, Notes on the treatment of oriental sore with berberine acid sulphate. *Indian med. Gaz.*, **LXIX.**, 72.

Chopra, R. N., Dikshit, B. B., and Chowhan, J. S., 1932, Pharmacological action of berberine. *Indian J. med. Res.*, **LIX.**, 1193.

Dostrowsky, A., 1929, Zur Behandlung der Leishmaniosis cutanea. *Arch. Schiffs- u. Tropenhyg.*, **XXXIII.**, 417.

Elias, S., 1936, Therapeutica da leishmaniose. *Hospital, Rio de Janeiro*, **II.**, 1011.

Gupta, B. M. Das, 1930, The treatment of oriental sore with berberine acid sulphate. *Indian med. Gaz.*, **LV.**, 683.

Gupta, B. M. Das, and Dikshit, B. B., 1929, Berberine in the treatment of oriental sore. *Indian med. Gaz.*, **LXIV.**, 67.

Hayward, E. W., 1933, Notes on berberine sulphate in oriental sore. *Indian med. Gaz.*, **LXVIII.**, 265.

Humphreys, R. M., and Mayne, F. S., 1935, Oral leishmaniasis in the Anglo-Egyptian Sudan. *Trans. R. Soc. Trop. Med. Hyg.*, **XXIX.**, 285.

Joannidès, N. Z., 1931, Neostibosan bei Leishmaniosis. *Dermat. Wschr.*, **XCII.**, 280.

Jolly, G. G., 1911, The treatment of oriental sore. *Indian med. Gaz.*, **XLVI.**, 466.

Karamchandani, P. V., 1927, An analysis of 337 cases of oriental sore treated by various methods. *Indian med. Gaz.*, **LXII.**, 558.

Kousa, 1935, Contribution au traitement du bouton d'Alep. *J. Egypt. med. Ass.*, **XVIII.**, 564.

Krasnjanskij, M. W., and Lawrow, A. P., 1936, Versuch einer Behandlung der Hautleishmaniose mit Gold. *Arch. Schiffs- u. Tropenhyg.*, **XL.**, 518.

Laqueur, B., 1932, Beitrag zur Therapie der Leishmaniosis cutanea. *Arch. Schiffs- u. Tropenhyg.*, **XXXVI.**, 489.

Laqueur, B., 1933, Ueber Chemotherapie der Leishmaniosis cutanea. *Arch. Schiffs- u. Tropenhyg.*, **XXXVII.**, 194.

Mazza, S., and Arias Aranda, C., 1931, Fuadinversuche bei der amerikanischen Leishmaniose. *Arch. Schiffs- u. Tropenhyg.*, **XXXV.**, 583.

Napier, L. E., 1927, " Kala azar," 2nd Edit. London.

Napier, L. E., and Haldar, K. C., 1930, The treatment of post-kala-azar dermal leishmaniasis. *Indian med. Gaz.*, **LXV.**, 371.

Photinos, G. T., 1930, Die Behandlung der Orientbeule durch lokale Injektionen mit Emetinum hydrochloricum. *Dermat. Wschr.*, **XCI.**, 1219.

Pützer, B., 1934, Das Berberin und seine Derivate in der Therapie der Orientbeule. *Med. u. Chem. Abh. med-chem. Forschungstätten. I. G. Farbenind.*, **II.**, 288.

SINCKE, G. E., 1935, Zwei erfolgreich mit dem kombinierten Arsen-Atimonpräparat Sdt 386B behandelte Fälle von Hautleishmaniose. *Arch. Schiffs- u. Tropenhyg.*, XXXIX., 63.

SORINSON, N. S., 1932, Beitrag zur Frage der Leimaniasis cutanea. Versuch der Behandlung mit Gold-präparaten. *Arch. Schiffs- u. Tropenhyg.*, XXXVI., 53.

VARMA, R. L., 1927, Berberine sulphate in oriental sore. *Indian med. Gaz.*, LXII., 84.

VIANNA, G., 1912, Sociedade Brasileira de Dermatologia, 4a Sessão Ordinaria. *Arch. brasil. Med.*, II., 422.

VIGNE, P., and FOURNIER, A., 1928, Le traitement du bouton d'Orient. À propos de quelques cas personnels. *Rev. prat. Mal. Pays chauds.*, VIII., 412.

WARMA, J. D., 1934, Further observations on the treatment of oriental sore. *Indian med. Gaz.*, LXIX., 616.

CHAPTER IV

THE CHEMOTHERAPY OF MALARIA

THE anti-malarial compounds at present in use comprise: (1) the four common cinchona alkaloids—quinine, quinidine, cinchonine and cinchonidine. (2) Cinchona derivatives, some of which, such as hydroquinine, are as active therapeutically as quinine. (3) Plasmoquine, atebrin and a number of other less well-known compounds of similar type. All the compounds now commonly employed in malaria therapy, including the natural alkaloids, have one factor in common: they are substitution derivatives of either quinoline or acridine. They may, however, be equally regarded as alkylamines or, in the case of the natural alkaloids, cyclic amines substituted by the quinoline or acridine nucleus. The inclusion of the quinoline or acridine nucleus may be looked upon as endowing them with an alkaloidal character, in that they are thus basic compounds in which at least one nitrogen atom forms part of a cyclic system (*cf.* Schmidt, 1932).

While the cinchona alkaloids are still the most widely used anti-malarial drugs, synthetic products such as plasmoquine and atebrin have received considerable attention, as a result of which it is now possible to define more precisely the part to be played by these products both in prophylaxis and in individual and mass treatment.

CINCHONA

The therapeutic properties of cinchona bark are generally believed to have been well known to the native Indians of Peru, who confided their knowledge to the Jesuits when missions were first established in the country after its conquest by Pizarro in 1527.

The first actual medicinal use of cinchona bark is supposed to have occurred in 1630 (*cf.* Wellcome, 1930), Don Juan López de Canizares, the Spanish corregidor of Loxa, being cured of an intermittent fever by an Indian cacique of Malactos, who revealed to him the curative properties of the bark and the proper way to administer it.

The bark was brought from Lima to Spain and Rome in 1632 by the Jesuit Barnabé de Cobo (1582–1657), who was Procurator of the Peruvian Province of the Order.

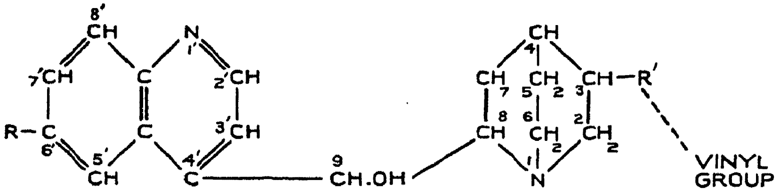
The word "Cinchona" was invented by Linnæus in 1742 for the genus of plants, the title being suggested by the titular name "Chinchon" of the Countess Francisca Henriquez de Ribera, the second wife of the Viceroy of Peru. Both the Count and Countess of Chinchon—the village still exists, or recently existed, in Spain—while in Lima suffered severely from fever, and in 1638 the former, an account of whose medical history has recently been found in Peru, was cured by the court physician, Juan de Vega, who administered cinchona bark.

The name quinaquina, quinquina, kinkina or kinakina has been derived from the Quichuan Indian language of Peru, in which re-duplication almost invariably implies the possession of medicinal qualities. In 1820 Pelletier and Caventou, in Paris, isolated from the bark two alkaloids which they named quinine and cinchonine. Cinchonidine and quinidine were isolated thirty-two years later.

THE ALKALOIDS OF CINCHONA

The four chief crystallisable alkaloids derived from cinchona bark are, as is well known, quinine, quinidine, cinchonine and cinchonidine, but over twenty other alkaloids have been isolated from various species of cinchona and cuprea. The majority of these alkaloids, the non-crystallisable and amorphous alkaloids, are sometimes described collectively as quinoidine: strictly speaking, however, quinoidine is the pitch-like residue left after removal of all the commercially valuable alkaloids from the total alkaloids of cinchona bark. The four alkaloids, quinine, quinidine, cinchonine and cinchonidine, form two pairs of isomerides of which each member of the first pair differs from each member of the second by the residue of a methoxyl group—CH_2O. In addition, the members of each pair yield for the most part the same products furnished by the two pairs from parallel series differing constantly by the residue of a methoxyl group—CH_2O.

The following general formula is now usually assigned to this group of alkaloids (*cf.* Rabe, 1909 ; Rabe, Huntenburg, Schultze and Volger, 1931).



Quinoline Group *Secondary Alcohol Group* *Quinuclidine Group.*

In quinine and quinidine $R = OCH_3$. $R' = CH : CH_2$.
In cinchonine and cinchonidine $R = H$. $R' = CH : CH_2$.
In cupreine $R = OH$. $R' = CH : CH_2$.
In the hydro bases R′ becomes $CH_2.CH_3$.
In the alkylcupreines R becomes OAlk (homologues of quinine).
In the alkylhydrocupreines and alkylhydrocupreidines R becomes OAlk and R′ becomes $CH_2.CH_3$ (homologues of dihydroquinine and dihydroquinidine).

It will be noticed that this formula includes four centres of asymmetry, numbered 3, 4, 8 and 9. The differences between the members of each pair of isomerides are due to differences in configuration at certain of these centres and, from a variety of evidence (*cf.* Lapp, 1935 ; Henry, Solomon and Gibbs, 1937 ; and Solomon, 1938), the direction of rotation at each of these centres for each of the principal cinchona alkaloids is now known and is given in the following table (Solomon, 1938) :—

STEREOCHEMICAL PICTURE OF THE CINCHONA ALKALOIDS

Substance.	Relative configurations at C atoms.	3	4	8	9
Cinchonine	dextro-rotatory series.				
Dihydrocinchonine					
Quinidine					
Dihydroquinidine					
Cinchonidine	lævo-rotatory series.				
Dihydrocinchonidine					
Quinine					
Dihydroquinine					

Modification of the cinchona alkaloids gives rise to a number of compounds of therapeutic interest, for with hydrogenation of the vinyl group and replacement of the methoxyl group of quinine and quinidine by higher alkyloxy groups the following compounds are produced. When the alkyloxy group is

$—OCH_3$	there is formed	Methylhydrocupreine.
$—OC_2H_5$		Ethylhydrocupreine (Optochin)
$—OCH(CH_3)_2$		*iso*Propylhydrocupreine.
$—OCH_2.CH(CH_3)_2$		*iso*Butylhydrocupreine.
$-OCH_2.CH_2.CH(CH_3)_2$		*iso*Amylhydrocupreine (Eukupin).
$).(CH_2)_5.CH(CH_3)_2$		*iso*Octylhydrocupreine (Vuzin).

Optochin has a curative action in pneumococcal infections in mice and is discussed more fully in relation to bacterial infections ; vuzin is toxic *in vitro* to *Corynebacterium diphtheriæ*, while eukupin and vuzin possess local anæsthetic properties.

A red azo dye (C. 77), prepared by Giemsa and Oesterlin (1933), has been tested in human malaria by Green (1934). This compound, 6′-methoxyquinoline-8′-azodihydrocupreine, was found to act like quinine on the parasites of sub-tertian and quartan malaria but to be inferior in benign tertian infections. It was, however, free from toxic effects and did not produce deafness or buzzing in the ears (*cf.* Missiroli and Mosna, 1934).

THE THERAPEUTIC EFFICIENCY OF THE CINCHONA ALKALOIDS AND THEIR DERIVATIVES

While the introduction of atebrin and plasmoquine represents a notable advance in the scientific treatment of malaria, quinine still ranks first in current practice, on account of its cheapness, clinical effectiveness, low toxicity and the widespread knowledge of its use and dosage.

The reasons why quinine should be preferred to the other alkaloids present in cinchona bark are not at first obvious, for the evidence obtained from clinical trials is by no means unanimous. MacGilchrist (1915), for instance, placed the therapeutic efficiency of the chief alkaloids in the following order of descending efficiency: dihydroquinine (hydroquinine) → quinine, quinidine, hydroquinidine → cinchonidine, hydrocinchonidine → cinchonine, hydrocinchonine.

Sinton and Bird (1929), on the other hand, regarded quinidine as inferior to quinine, cinchonidine and cinchonine in the treatment of benign tertian malaria in India.

Apart from clinical experiments on man, it is now possible to employ the method of testing anti-malarial drugs devised by Roehl (1926), in which the compounds to be examined are given by mouth to canaries infected with malaria. In using this technique it must, however, be remembered that individual birds may show considerable variation in their reactions to a particular drug, and that the effect of a particular compound on the parasites of bird malaria may not necessarily parallel that on the parasites of human malaria. "Dimeplasmin," for instance, is of little use in man though very active in avian malaria. In addition, it is essential that the alkaloids to be tested should be pure. Dawson and Garbade (1930), for instance, point out that commercial samples of cinchonidine may contain up to 10 per cent. of quinine together with some hydrocinchonidine.

Taking these considerations into account, Goodson, Henry and Macfie (1930) found that of the natural cinchona alkaloids, the most active in curing infections due to *Plasmodium inconstans* in canaries was dihydroquinine, followed by quinidine, quinine, cinchonidine and cinchonine in descending order. There is little to choose between the last four, though Giemsa and Oesterlin (1933) and Buttle, Henry and Trevan (1934) would rank quinidine rather lower in the therapeutic scale.

The effect of hydrogenation has been shown by Buttle, Henry and Trevan (1934) to vary greatly for different alkaloids. For quinine, and possibly quinidine, hydrogenation increases the activity, while for cinchonine the effect is probably to diminish the activity. If the anti-malarial activity of quinine in bird malaria be taken as 1, the therapeutic efficiency of the four main cinchona alkaloids and their dihydrogen derivatives is as follows :—

Dihydroquinine	1–2	Quinidine	Less than 0·5.
Quinine .	1	Cinchonine	Less than 0·2.
Dihydroquinidine .	0·5–1	Dihydrocinchonine .	,, ,,
Cinchonidine	About 0·5	Dihydrocinchonidine	,, ,,

The anti-malarial activity of certain *modified cinchona alkaloids* has also been tested. Quinine chloride was found by Giemsa *et al.* (1926) to be entirely lacking in activity; Cohen and King (1938) observed that the chloro-alkaloids, quinine chloride, hydroquinine chloride, quinidine chloride, hydroquinidine chloride, cinchonine chloride, cinchonidine chloride and hydrocinchonidine chloride, and Buttle, Henry and Trevan (1934) that dihydroquinine chloride and cinchonidine chloride were also inactive; these observations show that anti-malarial activity disappears in these alkaloids when the —CHOH— group is replaced by —CHCl—. A similar loss of activity is associated with its conversion into —CH_2— (desoxyquinine, desoxydihydrocinchonine, desoxydihydrocinchonidine, desoxycinchonine, desoxycinchonidine), CH— (quinine, hydroquinine and cinchine), —CO— (quininone) and CH.OAc— (acetyldihydroquinine) (*cf.* Giemsa and Oesterlin, 1933).

Although Giemsa and Oesterlin (1933) had shown that quinicine, an isomeride of quinine, is devoid of anti-plasmodial action, it seemed possible to Ainley and King (1938) that reduction to the carbinol, which occurs in two diastereoisomeric forms, might restore activity. Both *l*-dihydroquinicinol 2HCl and *d*-dihydroquinicinol 2HCl, however, proved inactive in bird malaria, as did α-*N*-methyldihydroquinicinol 2HCl.

Ainley and King (1938) also prepared 4-quinolyl-α-piperidylcarbinol, 4-(6-methoxyquinolyl)-α-piperidylcarbinol and their *N*-alkyl derivatives as hydrochlorides. No quinolyl derivative without the 6-methoxy group had any activity. *iso*-4-(6-Methoxyquinolyl)-α-piperidylcarbinol and 4-(6-methoxyquinolyl)-α-piperidylcarbinol, however, were both active in bird malaria and appear to be the first compounds to be synthesised on the pattern of the quinine molecule with anti-malarial activity. The toxicity of this whole group of substances is comparable with the toxicity of the cinchona alkaloids and not with substances of the type of plasmoquine, which are much more toxic.

The activity of *epi*-C^9-bases was first investigated by Dirscherl and Thron (1935), who found a loss of activity. Epimerisation at C^9 (*cf.* Formula I.) was shown by Buttle *et al.* (1938) to reduce the activity of quinine from 1 to 0·1 in *epi*-C^9-quinine and that of quinidine from 0·5 to 0·1 in *epi*-C^9-quinidine ; whilst dihydroquinine has a quinine ratio of 1·35, *epi*-C^3-dihydroquinine has the same activity as quinine.

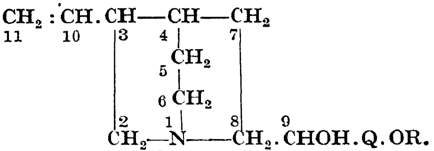
No obvious relationship can be traced between optical activity and anti-malarial action in the cinchona alkaloids, for while the *l*-bases, quinine and cinchonidine, are more active than the *d*-bases, quinidine and cinchonine (Buttle *et al.* 1934), *apo*quinidine methyl ether (+ 334·2°) is more active than either of its *l*-isomerides, α- or *β-iso*quinine. The conversion of the phenolic *l*-bases, *apo*quinine and *iso-apo*quinine, into their methyl ethers, *β*- and *α-iso*quinines, results in a reduction of anti-malarial action ; the converse is the case when the *d*-phenolic base, *apo*quinidine, is methylated.

The anti-malarial activities of the *alkylquitenines* have been investigated by Goodson, Henry and Macfie (1930). On oxidation of quinine the inactive acid quitenine is formed. Activity is restored to quitenine and the other analogous carboxylic acids derived from quinidine, cinchonidine and cinchonine by esterification. As the molecular weight of the alcohol used for esterification increases, so does the anti-malarial activity, the maximum being reached at butyl or amyl quitenine, after which there is a fall.

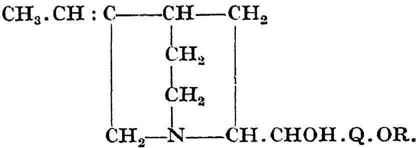
Cohen and King (1938) showed that methylquitenine dihydrochloride was slightly active, thus confirming the results obtained by Goodson, Henry and Macfie (1930) ; methyl cinchotenine and methylcinchotenidine dihydrochloride were inactive therapeutically, as were quitenamide and quitenmethylamide, 2-methyl-4-(3-pyridino) quinoline 2HCl and 2-styryl-4-*β*-pyridino-quinoline 2HCl.

Since the alkylquitenines are easily hydrolysed, Buttle, Henry *et al.* (1938) prepared a series of *apo*quinine and *apo*quinidine ethers. These complex compounds have been investigated chemically by Henry *et al.* (1934, 1935 and 1937).

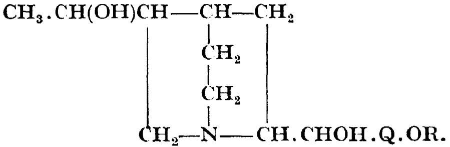
When a series of ethers of the phenolic base *apo*quinine (Formula II., R = H) and of its reduction product dihydrocupreine (Formula IV., R = H) were tested therapeutically in canaries it was found that if each series is divided into members containing odd and even numbers of carbon atoms there is, with some anomalies, a definite tendency in the four series so produced for the quinine ratio to rise to a maximum and then to fall as the series is ascended :—



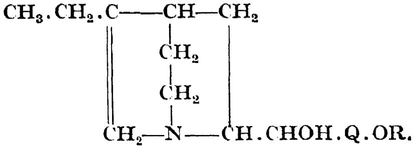
(Formula I.)



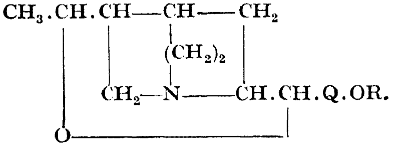
(Formula II.)



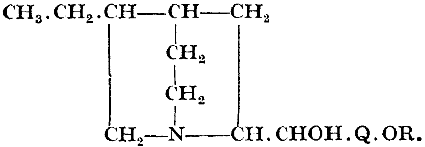
(Formula III.)



(Formula IV.)



(Formula V.)



(Formula VI.)

Q = Quinolyl residue.

R = Alkyl group or hydrogen.

A similar curious alternation in the parasiticidal activity of members of a homologous series was first noted by Magidson *et al.* (1933, 1935 and 1936) in testing the anti-malarial activity in infected siskins of a series of 6-methoxyquinolines substituted in position 8 (*cf.* Formula I.) by the chain $—NH—[CH_2]_n—NEt_2$, the chemotherapeutic indices (i) for different values of n being :—

n		3	4	5	6	7	8	9	10	11
i		26	—	25		34		40		5
i	6	—	11		13	—	—	—	—	—

This alternation was confirmed by Bovet *et al.* (1934), who tested the same series of compounds on the Java sparrow, *Padda oryzivora.* It was also observed that as the series was ascended the drugs acquired schizontocidal action in addition to their original gametocidal character.

Alternation, however, is not always observed in such tests with homologous series, for Magidson and Strukow (1933[2]) found that when the chain in position 8 was kept constant at $—NH—[CH_2]—NEt_2$ and the group in 6 varied from HO— to $C_5H_{11}O$—, there was a continuous but irregular fall in the chemotherapeutic index from 1 : 13 to 0. Similarly, Magidson and Grigorowsky (1936) showed that in a short series of substituted acridines with methoxyl at position 7, chlorine at position 3, and at position 5 the chain

ANTI-MALARIAL ACTIVITY IN HOMOLOGOUS SERIES OF AND DIHYDROCUPREINE ETHERS.
(Buttle *et al.* 1938).

Quinine ratio.		*ethers.*	*Name of ether.*	*Dihydrocupreine ethers.*	*Quinine ratio.*	
Even.	Odd.				Odd.	Even.
0·69	—	$C_{20}H_{24}O_2N_2$	Methyl	$C_{20}H_{26}O_2N_2$	—	1·35
—	1·18	$C_{21}H_{26}O_2N_2$	Ethyl	$C_{21}H_{28}O_2N_2$	1·05	—
1·23	—	$C_{22}H_{28}O_2N_2$	*n*-Propyl	$C_{22}H_{30}O_2N_2$	—	1·49
—	1·10	$C_{23}H_{30}O_2N_2$	*n*-Butyl	$C_{23}H_{32}O_2N_2$	1·87	—
1·47	—	$C_{24}H_{32}O_2N_2$	*n*-Amyl	$C_{24}H_{34}O_2N_2$		
—	1·72	$C_{25}H_{34}O_2N_2$	*n*-Hexyl	$C_{25}H_{36}O_2N_2$	1·50	
1·21	—	$C_{26}H_{36}O_2N_2$	*n*-Heptyl	$C_{26}H_{38}O_2N_2$		1·70
—	1·6	$C_{27}H_{38}O_2N_2$	*n*-Octyl	$C_{27}H_{40}O_2N_2$	1·43	—
0·98	—	$C_{28}H_{40}O_2N_2$	*n*-Nonyl	$C_{28}H_{42}O_2N_2$		1·12
—	1·18	$C_{29}H_{42}O_2N_2$	*n*-Decyl	$C_{29}H_{44}O_2N_2$	1·6	—
0·75	—	$C_{30}H_{44}O_2N_2$	*n*-Undecyl	$C_{30}H_{46}O_2N_2$		1·25
—	0·58	$C_{35}H_{54}O_2N_2$	Cetyl			

—NH—$[CH_2]_n$—NEt_2 where $n = 2$ to 6, the chemotherapeutic index began at 1 : 8, rose to 1 : 20 at $n = 4$ and fell to 1 : 6 and 1 : 5 at $n = 5$ and 6 respectively.

These observations show that changes in action *in vivo* of a homologous series of parasiticidal drugs cannot yet be satisfactorily correlated with chemical structure, solubility or other known physical factors. Possibly it is a combination of factors which leads to alternation in the effect of length of chain on activity.

A study of the anti-malarial activity of a short series of *dihydrocupreidine ethers* (Formula VI. : R = Alkyl) by Buttle *et al.* (1938) shows that in the dextrorotatory bases a rise in activity, with a maximum at the ethyl compound, occurs as the side chain is increased in length. In the case of the hydroxy*apo*-bases (Formula III. : R = H or Me) anti-malarial activity is reduced, since the presence of a phenolic hydroxyl group in the quinoline nucleus and an additional hydroxyl group attached to carbon atom 10 reduce the basic character which seems to be essential for anti-malarial action. Niquine was found by Buttle *et al.* (1938) to be less active than quinine, but niquidine, *iso*niquidine and dihydroniquidine had quinine ratios of 1·45, 1·05 and 1·06 respectively, niquidine being much the most active of the dextrorotatory cinchona alkaloids yet known.

It is obvious that at present no precise general conclusions can be drawn as to the mode of action of the cinchona alkaloids as anti-malarial drugs. It is, however, generally agreed that though the reticulo-endothelial system may play some part in the process (*cf.* Kritschewski and Demidowa, 1934), their action on the parasites is a direct one, and that any reduction in their basic character, and this applies also to other synthetic anti-malarial drugs, is associated with a reduction or total disappearance of activity. Fulton (1937) was unable to determine any relationship between anti-malarial action and the distribution of cinchona alkaloids between red cells and serum. Inactivity may be caused by small structural changes, especially in the —CHOH— group which links up the two parts of the cinchona alkaloids and by changes in spatial distribution. These findings, and especially the alteration in activity shown by successive members of homologous series, support the view of Buttle and his colleagues (1938) that the essential condition for anti-malarial activity is a close "fit" between the drug and the protein molecules of the parasites.

Fischl and Singer (1935) showed that a number of fluorescent dyes, including atebrin, were fixed by the plasmodia of bird malaria; Oesterlin (1936) therefore believes that the parasiticidal action of dyes in malaria is associated with fluorescence. While the presence of a strong basic group is necessary for fixation of the dye, the subsequent lethal action, it is suggested, is associated with the properties causing fluorescence.

Kritschewski and Demidowa (1934) believe that the activity of malarial drugs is greatly reduced by blocking the reticulo-endothelial system with trypan blue.

References

AINLEY, A. D., and KING, H., 1938, Antiplasmodial action and chemical constitution. Part II. Some simple synthetic analogues of quinine and cinchonine. *Proc. roy. Soc.*, B., CXXV., 60.

BOVET, D., BENOIT, G., and ALTMAN, R., 1934, Action thérapeutique de quinoléines à poids moléculaire élevé, homologues de la plasmoquine, sur les hématozoaires des calfats et des serins. *Bull. Soc. Path. exot.*, XXVII., 236.

BUTTLE, G. A. H., HENRY, T. A., SOLOMON, W., TREVAN, J. W., and GIBBS, E. M., 1938, The action of the cinchona and certain other alkaloids in bird malaria. III. *Biochem. J.*, XXXII., 47.

BUTTLE, G. A. H., HENRY, T. A., and TREVAN, J. W., 1934, The action of the cinchona and certain other alkaloids in bird malaria. II. *Ibid.*, XXVIII., 426.

COHEN, A., and KING, H., 1938, Antiplasmodial action and chemical constitution. Part I. Cinchona alkaloidal derivatives and allied substances. *Proc. roy. Soc.*, B., CXXV., 49.

DAWSON, W. T., and GARBADE, F. A., 1930, Idiosyncrasy to quinine, cinchonidine and ethylhydrocupreine and other lævorotatory alkaloids of the cinchona series. *J. Amer. med. Ass.*, XCIV., 705.

DIRSCHERL, W., and THRON, H., 1935, Das Vorkommen von Epi-chinin und Epichinidin in der Chinarinde. Ein Beitrag zur Chemie des Chinoidins. *Liebigs. Ann.*, DXXI., 48.

FISCHL, V., and SINGER, E., 1935, Die Wirkungsweise chemotherapeutisch verwendeter Farbstoffe. *Z. Hyg. InfektKr.*, CXVI., 348.

FULTON, J. D., 1937, Studies in the chemotherapy of malaria. The distribution of antimalarial drugs between red cells and serum. *Ann. trop. Med. Parasit.*, XXXI., 7.

GIEMSA, G., and OESTERLIN, M., 1933, Chemotherapeutische Studien auf dem Gebiete der Chinaalkaloide. *Arch. Schiffs- u. Tropenhyg.*, XXXVII., Beih. 4, 217.

GIEMSA, G., WEISE, W., and TROPP, C., 1926, Chemotherapeutische Studien mit Vogelmalaria (*Plasmodium præcox*). *Ibid.*, XXX., 334.

GOODSON, J. A., HENRY, T. A., and MACFIE, J. W. S., 1930, The action of the cinchona and certain other alkaloids in bird malaria. *Biochem. J.*, **XXIV.**, 874.

GREEN, R., 1934, Lectures on the development and use of the synthetic antimalarial drugs. *Bull. Inst. med. Res. Fed. Malay States*, No. 2.

HENRY, T. A., and SOLOMON, W., 1934, Modified cinchona alkaloids. Part I. Apoquinine and apoquinidine. *J. chem. Soc.*, 1923.

HENRY, T. A., SOLOMON, W., and GIBBS, E. M., 1935, Modified cinchona alkaloids. Part II. The action of sulphuric acid on quinine and quinidine. *Ibid.*, 966.

HENRY, T. A., SOLOMON, W., and GIBBS, E. M., 1937, Modified cinchona alkaloids. Part IV. Constitution. *Ibid.*, 592.

KRITSCHEWSKI, I. L., and DEMIDOWA, L. W., 1934, Ueber eine noch unbekannte Funktion des retikuloendothelialen Systems. XXII. Ueber die Bedeutung des retikuloendothelialen Systems in der Therapie der Malaria. *Z. ImmunForsch.*, **LXXXIV.**, 14.

LAPP, C., 1935, Le pouvoir rotatoire spécifique des sels de quinine, quinidine, cinchonine et cinchonidine. *C. R. Acad. Sci., Paris*, **CCI.**, 80.

LINNÆUS, C., 1742, " Genera Plantarum," 2nd Edit.

MACGILCHRIST, A. C., 1915, The relative therapeutic value in malaria of the cinchona alkaloids—quinine, cinchonine, quinidine, cinchonidine, quinoidine and the two derivatives, hydroquinine and ethylhydrocupreine. *Indian J. med. Res.*, **III.**, 1.

MAGIDSON, O. J., and GRIGOROWSKY, A. M., 1936, Acridin-Verbindungen und ihre Antimalaria-Wirkung (I. Mitteil). *Ber. dtsch. chem. Ges.*, **LXIX.**, 396.

MAGIDSON, O. J., MADAJEWA, O. S., and RUBZOW, M. W., 1935, Die Derivate des 8-Aminochinolins als Antimalariapräparate. Mitt. IV : Verbindungen mit langen Ketten in 8-Stellung. *Arch. Pharm. Berl.*, **CCLXXIII.**, 320.

MAGIDSON, O. J., and STRUKOW, I. TH., 1933[1], Die Derivate des 8-Aminochinolins als Antimalariapräparate. Mitt. I : Die Wirkung von Alkyl in Stellung 6 auf chemotherapeutische Eigenschaften. *Ibid.*, **CCLXXI.**, 359.

MAGIDSON, O. J., and STRUKOW, I. TH., 1933[2], Die Derivate des 8-Aminochinolins als Antimalariapräparate. Mitt. II : Der Einfluss der Länge der Kette in Stellung 8. *Ibid.*, **CCLXXI.**, 569.

MISSIROLI, A., and MOSNA, E., 1934, Sulle proprietà terapeutiche di un prodotto derivato della Chinina : C. 77-Giemsa. *Riv. Malariol.*, **XIII.**, 148.

OESTERLIN, M., 1936, Chemotherapie, Fluorescenz und Krebs. *Klin. Wschr.*, **XV.**, 1719.

PELLETIER and CAVENTOU, 1820, Analyse chimique de quinquinas suivie d'observations médicales sur l'emploi de la quinine et de la cinchonine. *Ann. Chim. (Phys.)*, **XV.**, 291, 1337.

RABE, P., 1909, Zur Kenntniss der Chinaalkaloide. *Liebigs Ann.*, **CCCLXV.**, 353.

RABE, P., HUNTENBURG, W., SCHULTZE, A., and VOLGER, G., 1931, Die Totalsynthese der China-Alkaloide, Hydrochinin und Hydrochinidin. *Ber. dtsch. chem. Ges.*, **LXIV.**, 2487.

ROEHL, W., 1926, Die Wirkung des Plasmochins auf die Vogelmalaria. *Arch. Schiffs- u. Tropenhyg.*, **XXX.**, Beih. 2, 311.

SCHMIDT, J., 1932, A text-book of organic chemistry. (English translation.) London.

SINTON, J. A., and BIRD, W., 1929, Studies in malaria with special reference to treatment. Part XI. The cinchona alkaloids in the treatment of benign tertian malaria. *Indian J. med. Res.*, XVI., 725.

Solomon, W., 1938, Modified cinchona alkaloids. Part V. β-*iso*-Quinotoxin and the stereochemistry of the parent bases. *J. chem. Soc.*, 6.

Wellcome, H. S., 1930, "Souvenir, Cinchona Tercentenary Celebration and Exhibition." London.

OXYQUINOLINE DERIVATIVES

Under the name of "Paludex," a compound described as "copper oxyquinoline sulphonate of soda" has recently been used for the treatment of malaria, largely by Belgian workers. Paludex is a greenish amorphous powder freely soluble in water, with a neutral reaction and stable under ordinary conditions of temperature and humidity. Van Nitsen (1936 and 1937) and van Nitsen and Serra (1936) have used the drug in the treatment of a considerable number of cases of malaria, chiefly due to *P. falciparum*, both in Europeans and African natives. The dose employed for an adult is 1 gm. on the first day, 1·20 gm. on the second day, and an increase of 0·20 gm. a day till the sixth day, when 2 gm. are given. This last dose can be continued for some days. Both schizonts and gametocytes of all species of parasite are said to disappear rapidly from the peripheral blood-stream, and in only six out of 163 patients had treatment to be continued for more than seven days. No symptoms of intolerance were noted, and in cases where there was much vomiting the drug was often well retained and absorbed. The drug has been found to have a very definite tonic effect. Sufficient observations have not yet been made to determine the frequency of relapse. Small doses of 0·5 gm. of quinine combined with 0·6 to 1 gm. of paludex a day are said to be more effective than the normal doses of either drug taken alone. In bird malaria (*P. cathemerium*), halteridium (*Halteridium oryzivoræ*) infections and monkey malaria (*P. knowlesi* and *P. gonderi*), Rodhain and Hendrix (1937) were unable to demonstrate any favourable action. Further observations on human malaria in endemic and hyperendemic areas are obviously required before the value of paludex can be estimated.

References

RODHAIN, J., and HENDRIX, H., 1937, Essais de traitement du paludisme des oiseaux et des singes au moyen du "paludex." *Bull. Inst. colon. belge*, VIII., 137.

VAN NITSEN, R., 1936, Le traitement de la malaria par le paludex, nouveau dérivé quinoléinique (étude basée sur 1000 observations d'indigènes). *Ann. Soc. belge Méd. trop.*, XVI., 387.

VAN NITSEN, R., 1937, Contribution à la chimiothérapie du paludisme. *Brux. méd.*, XVII., 587.

VAN NITSEN, R., and SERRA, D., 1936, Le paludisme chez les européens traités par le paludex. *Ann. Soc. belge Méd. trop.*, XVI., 409.

DIHYDROQUINAMINE

A number of observers such as Charron (1933) and Krouch (1934) have from time to time claimed that dihydroquinamine preparations have an action both on gametocytes and schizonts. Tara (1933) and more recently Sautet (1937), have, however, failed to confirm these claims. When injected intramuscularly in 5 c.c. amounts containing dihydroquinamine uranyl hydrochloride 0·01 gm., dihydroquinamine benzylene ethyl arsanilate 0·05 gm., quinine hydrochloride 0·35 gm. and urethane 0·185 gm., parasites rapidly disappeared and the temperature fell, but, on the other hand, the nausea and gastralgia were so severe as to prevent the continuation of treatment. By mouth the action of dihydroquinamine on the parasites was far less easily demonstrable.

References

CHARRON, E., 1933, Sur un nouveau dérivé de la quinine à action gamétocide. *Rev. Méd. Hyg. trop.*, XXV., 59.

KROUCH, M., 1934, La chimiothérapie antipaludéenne. Essai d'un complexe gaméticide (CG) en milieu rural tunisien. *Bull. Soc. Path. exot.*, XXVII., 141.

SAUTET, J., 1937, À propos du traitement du paludisme par les dérivés de la dihydroquinamine. *Bull. Soc. Path. exot.*, XXX., 461.

TARA, S., 1933, Sur l'emploi de la dihydroquinamine. *Rev. Méd. Hyg. trop.*, XXV., 1.

THE TREATMENT OF HUMAN MALARIA

The work that has been carried out in the past few years on cinchona derivatives and the newer synthetic products has emphasised the many factors which must be taken into account if satisfactory results are to be obtained in treatment. Thus: (1) each drug has special indications applicable to its parenteral administration, (2) the species of parasite, and more especially the strain of the particular parasite, also influences materially the response to the drug. Indeed James (1932) states that from the chemotherapeutic point of view the biological susceptibility of a parasite to the action of drugs may be of more importance than its morphological characters. A number of instances of the differing susceptibility of varying strains of the same species are given in the Fourth Report of the League of Nations Malaria Commission (1937). (3) As a result of defective intestinal absorption certain individuals may fail to respond to certain drugs (*cf.* Chopra, Sen and Roy, 1937). (4) Treatment must not interfere with the acquirement of tolerance. In the case of therapeutically induced malaria, there is some evidence in favour of the view that better results are obtained in treatment, if the infection is allowed to continue for some time.

Certain observers, such as Ashford (1936), believe that intensive collective treatment may seriously interfere with the acquirement of tolerance, more especially in indigenous children, in whom reinfection is probable if not inevitable. There is, however, no conclusive evidence to show that in therapeutic doses any of the generally employed chemotherapeutic drugs interferes with the development of tolerance.

A consideration of the above factors does, however, go far to explain the contradictory conclusions drawn by observers in different countries in regard to the curative, and more especially the prophylactic, value of quinine and the synthetic antimalarials.

CINCHONA ALKALOIDS IN THE TREATMENT OF HUMAN MALARIA

The action of cinchona alkaloids and of atebrin and plasmoquine in the treatment of malaria has recently been fully discussed in the Fourth Report of the Malaria Commission of the League of

Nations (1937): as a result only a brief summary will here be given. Quinidine sulphate has been used in doses of 0·6 gm. a day in the treatment of both benign and malignant tertian infections by Sanders (1935), and is of value in cases of hypersensitiveness to quinine; Stone *et al.* (1933) have also employed hydrocinchonidine and hydrocinchonine in daily doses of 0·5 gm. "Quinimax," a preparation containing the hydrochloride of quinine, quinidine, cinchonine and cinchonidine, with resorcin and sodium chloride, is said to cause no reaction when injected intramuscularly (*cf.* Ragiot and Moreau, 1937). The cinchona compounds generally administered are quinine and totaquina or cinchona febrifuge.

(*a*) *Quinine.* In order to cause the disappearance of the trophozoites of *Plasmodium vivax* from human blood during a primary infection, the Fourth Report of the Malaria Commission of the League of Nations (1937) recommends a mean daily dose of 1 gm. of quinine hydrochloride for from five to seven days. With such treatment the trophozoites disappear on an average on the third day and do not reappear until after a latent period of varying length in the course of the primary relapse. A minimum daily dose of 0·5 gm. of quinine hydrochloride is sometimes sufficient to cause a temporary disappearance of trophozoites. In quartan infections due to *P. malariæ* the same effects are usually obtained, but in infections with *P. falciparum* the average daily dose must be fixed at about 1·30 gm., while with certain strains of greater virulence it is necessary to use 2 gm. to obtain a rapid effect on the parasites. With the usual dose of 1 gm. the trophozoites of *P. falciparum* disappear on an average one day later than in the case of *P. vivax.* Quinine, in the doses indicated, exercises its parasiticidal activities on the young forms of *P. vivax* and *P. malariæ* capable of producing gametocytes as well as on fully developed gametocytes, though the action on the latter is very slow (*cf.* Parrot *et al.*, 1937). In the case of *P. falciparum* the formation of the pregametocytes is but little affected. Quinine may thus be regarded as directly schizontocidal and indirectly gametocidal in the case of *P. falciparum.*

Although quinine undoubtedly reduces the number of relapses, their frequency is influenced by individual factors and by the strain of parasite. The treatment of primary infections due to *P. vivax* or *P. malariæ* infections with daily doses of 1 gm. is followed by relapses in a proportion of individuals which may be as high as 50 per cent. Ascoli (1936 and 1937) and a number of Italian workers have associated injections of adrenalin with quinine. From 0·01 to 0·1 mgm. of adrenalin is given subcutaneously and is thought to be of value in chronic cases of malaria, more especially in reducing the size of the spleen. Acaprine has been used for a similar purpose (Radvan and Alexandrescu, 1937).

When applied in each attack quinine has proved to be of efficacy in reducing splenomegaly in endemic regions, particularly among children. Its action, however, is somewhat transient if the community concerned is subject to a high relapse rate or is exposed to frequent reinfection.

Quinine cannot be regarded as a true causal prophylactic, and in preventing induced infection it appears to be less effective than atebrin (*cf.* James, 1933, and Ciuca, Ballif, Chelarescu, Isanos and Glaser, 1937). In clinical prophylaxis opinions differ greatly in regard to the value of quinine. Orenstein (1929), for instance, regards quinine as of little value for mass prophylaxis, while Bispham (1936) believes that it is far less efficient than atebrin. In Algeria, however, Parrot, Catanei and Ambialet (1937) found that quinine hydrochloride (0·1 to 0·4 gr. daily) was, if anything, superior to daily doses of 25 to 500 mgm. of atebrin. The Malaria Commission of the League of Nations points out that very useful results can be obtained with daily doses of quinine (0·4 gm.) given during the whole of the malaria transmission season and even for a few weeks longer.

The chief advantage of quinine is, however, that it requires no such close supervision as is necessary in the case of atebrin, nor is it so likely to cause toxic reactions in children, among whom gametocyte carriers are more numerous than among adults living in the same environmental conditions.

One of the main benefits of quinine is that in the usual doses it does not affect the patient's general condition adversely, does not hinder the process of immunisation and has, as a rule, few toxic effects except in those rare persons who are sensitive to quinine. Mühlens (1937) believes that this idiosyncrasy may be inherited.

Although the exact method of action of quinine on malarial plasmodia is still unknown, Lourie (1934) has brought forward evidence to show that quinine acts by inhibiting reproduction, retarding the asexual cycle and decreasing the number of merozoites. The mechanism is thus quite distinct from that of acquired immunity where the parasites are killed without any evidence that the rate of reproduction is inhibited. Lourie (1935) has failed to find evidence that the prolonged administration of quinine or plasmochin leads to drug resistance on the part of the plasmodia.

References

Ascoli, M., 1936, Sulla cura della infezione malarica cronica. *Riforma med.*, XLII., 351.

Ascoli, M., 1937, Tatsachen und Fragestellungen meiner neuen Malariatherapie. *Münch. med. Wschr.*, LXXXIV., 370.

Ashford, M., 1936, The nature of immunity to malaria in relationship to anti-malarial therapy. *Amer. J. trop. Med.*, XVI., 665.

Bispham, W. N., 1936, A report on the use of atabrine in the prophylaxis of malaria. *Ibid.*, XVI., 547.

Chopra, R. N., Sen, B., and Roy, A. C., 1937, Individual variation in the effectiveness of synthetic antimalarial drugs (a preliminary note). *Indian med. Gaz.*, LXXII., 131.

Ciuca, M., Ballif, L., Chelarescu, M., Isanos, M., and Glaser, L., 1937, On drug prophylaxis in therapeutic malaria. *Trans. R. Soc. trop. Med. Hyg.*, XXXI., 241.

James, S. P., 1932, Synthetic antimalarial remedies and quinine. *Ibid.*, XXVI., 105.

James, S. P., 1933, Anti-malarial chemotherapeutic tests at Devon Mental Hospital, *Ibid.*, XXVII., 289.

Lourie, E. M., 1934, Studies on chemotherapy in bird malaria. II. Observations bearing on the mode of action of quinine. *Ann. trop. Med. Parasit.*, XXVIII., 255.

Lourie, E. M., 1935, Studies on chemotherapy in bird malaria. IV. Failure to promote drug resistance in *Plasmodium cathemerium* by prolonged administration of quinine or plasmochin. *Ibid.*, XXIX., 421.

Mühlens, P., 1937, Chinin-Nebenwirkungen. *Arch. Schiffs- u. Tropenhyg.*, XLI., 710.

Orenstein, J. A., 1929, Can quinine distribution be expected to reduce malaria in South Africa. *J. med. Ass. S. Afr.*, III., 479.

Parrot, L., Catanei, A., and Ambialet, R., 1937, Comparative experiments in mass prophylaxis of malaria by means of quinine and of synthetic drugs. *Bull. Hlth. Organ. League of Nations*, VI., 683.

Radvan, I., and Alexandrescu, D., 1937, Essai de thérapeutique des splénomégalies paludéennes par l'acaprine. Réduction rapide des splénomégalies palustres par l'injection d'acaprine. *Bull. Soc. Path. exot.* XXX., 362.

RAGIOT, C., and MOREAU, P., 1937, Essais thérapeutiques d'une nouvelle formule à base d'alcaloides totaux du quinquina. *Rev. Méd. Hyg. trop.*, XXIX., 85.

SANDERS, J. P., 1935, Treatment of malaria with a short course of quinidine. *Amer. J. trop. Med.*, XV., 651.

STONE, C. T., GASKILL, R. C., SANDERS, J. P., BARTON, J. C., SCHULZE, V. E., and DAWSON, W. T., 1933, Hydrocinchonidine and hydrocinchonine in malaria. *Ibid.*, XIII., 437.

TOTAQUINA

The fact that the alkaloids of cinchona, other than quinine, possess therapeutic activities almost as great as quinine itself has increased the possibility of using total extracts of cinchona bark. Such extracts, which are obviously less expensive than the purified alkaloids, were first prepared in India in 1874 at the instance of Dr. J. E. de Vrij, who suggested the manufacture of a powder containing all the cinchona alkaloids derived from the red bark of *Cinchona succirubra*, in which quinine constitutes about one-third of the alkaloids present. This powder was called quinetum or febrifuge. By 1903 trees of *Cinchona succirubra* had become very rare in India since, owing to the idea that quinine was the only cinchona alkaloid of value in malaria, attention had been focussed on the cultivation of *C. calisaya* and *C. ledgeriana* and their hybrids, species yielding yellow bark in which the proportion of quinine to the other alkaloids is about 70 per cent. *C. calisaya* and *C. ledgeriana*, though they can be raised with care in the eastern Himalayas, grow with ease only in their natural home in Bolivia and in Java. Cinchona febrifuge was therefore manufactured from the alkaloids which remained after the extraction of quinine from yellow bark, some quinine being added to make it more or less similar in composition to the original febrifuge. It is, therefore, not surprising that somewhat variable results were obtained in the treatment of malaria with this relatively unstandardised febrifuge. In order to overcome this difficulty the Malaria Commission of the League of Nations (1932) introduced an improved and standardised mixture known as "Totaquina." This mixture must consist of 70 per cent. of cystallisable alkaloids and not less than 15 per cent. of quinine: on the other hand, it should contain not more than 20 per cent. of amorphous alkaloids and not more than 5 per cent. of water, while the mineral ash must not exceed 5 per cent. and, if possible, 3 per cent. Two types of totaquina are allowed: Type I is prepared from the total alkaloids of *C. succirubra*, and if it is to be produced on a large scale it will necessitate the extensive planting of trees of this species. Type II, in which there is as a rule more cinchonine than in Type I, is prepared from *C. ledgeriana* residues with the addition of sufficient quinine to bring it up to specification. Although there have been from time to time complaints that totaquina is not completely soluble in an acid mixture and undoubtedly complete standardisation of composition cannot be obtained, nevertheless there is general agreement (*cf.* Pampana and Fletcher, 1934) that totaquina acts like quinine as a potent remedy in all forms of malaria. Hicks and Chand (1935) in India found that both types of totaquina were equally efficacious, provided daily doses of 0·6 gm. per 70 kilogram of body weight were given in benign tertian and 1·2 gm. per 70 kilogram of body weight in subtertian infections. In Roumania, Parvulescu *et al.* (1934) and Slatineanu *et al.* (1934) found Type II less efficient than Type I unless larger doses were given, while in the Malay States, Field (1934) found that with Type II totaquina the parasites disappeared rather more slowly than with quinine. In the Philippines, Maranon, Perez and Russell (1935) found a locally-produced Type II almost as efficient as quinine sulphate. While totaquina possesses no advantage therapeutically over quinine and is, if anything, slightly less efficient, nevertheless its cost of production is so much lower that its use is obviously indicated in countries such as India where, as Russell (1937) points out, at least 100 million persons a year suffer from malarial infection. Since the adoption of totaquina by the British Pharmacopœia (1933) its use has become widespread. In the Philippines, as in India, an excellent totaquina of type I has been prepared from the total alkaloids extracted from the bark of either *C. succirubra* or *C. robusta* (Ejercito and Santos, 1937).

References

EJERCITO, A., and SANTOS, G. O., 1937, The Philippine totaquina in the treatment of human malaria : preliminary report. *Mon. Bull. Manila Hlth. Bur.*, XVII., 219.

FIELD, J. W., 1934, Notes on totaquina. *Malaya med. J.*, IX., 26.

HICKS, E. P., and CHAND, D., 1935, The relative clinical efficacy of totaquina and quinine. *Rec. Malar. Surv. India*, V., 39.

MARANON, J., PEREZ, A., and RUSSELL, P. F., 1935, Philippine totaquina. *Philipp. J. Sc.*, LXI., 229.

PAMPANA, E. J., and FLETCHER, W., 1934, The therapeutic efficacy of totaquina in human malaria. I. Clinical tests carried out under the auspices of the Malaria Commission. II. Critical analysis of the results achieved. *Quart. Bull. Hlth. Org., League of Nations*, III., 325.

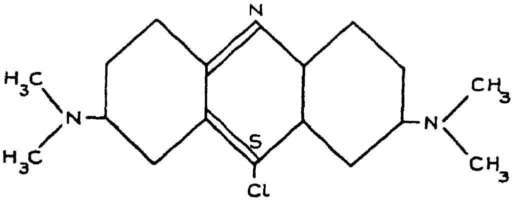
PARVULESCU, CONSTANTINESCO, N., and BOERIO, V., 1934, Efficacité comparée du totaquina dans le paludisme humaine (infection naturelle). *Arch. roum. Path. exp. Microbiol.*, VII., 523.

RUSSELL, A. J. H., 1937, Quinine supplies in India. *Rec. Malar. Surv. India*, VII., 233.

SLATINEANU, A., CIUCA, M., BALTEANU, I., ALEXA, E., ALEXA, I., FRANCKE, M., and RUGIÑA, I., 1934, Efficacité thérapeutique des alcaloïdes totaux de l'écorce de quinquina dans le paludisme humain (infection naturelle). *Bull. Soc. Path. exot.*, XXVII., 723.

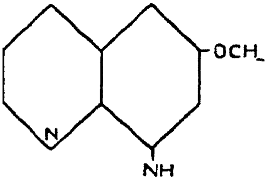
PLASMOQUINE

During the war shortage of quinine led to experiments at Elberfeld, the object of which was to obtain some synthetic drug which could be used in place of the naturally occurring cinchona alkaloids. Apart from the arsenicals the only compound then known to be active in malaria was methylene blue, the value of which had been demonstrated in 1891 by Guttman and Ehrlich.



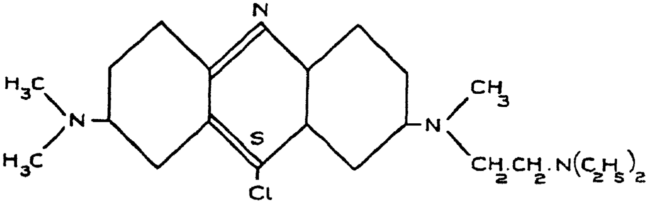
Methylene blue.

Replacement of one of the NMe_2 groups of methylene blue by the chain $NMe.CH_2.CH_2.N(C_2H_5)_2$ led to the formation of the following compound which (*cf.* Schulemann, 1932), in its turn, was found to be active in bird malaria. Thereupon the methylene blue complex was discarded and replaced by quinoline, the amino derivative of which had also been found to have a very slight



Quinoline.

therapeutic effect in bird malaria (Schulemann, Schönhöfer and Wingler, 1932). The resulting compound was plasmoquine, originally termed "beprochin," "plasmoquin," and later, in English speaking countries, "plasmoquin" or "plasmoquine." In France the same compound is sometimes termed "præquine." The constitution was stated by Hörlein (1926) to be 6-methoxy-8-diethylamino-*iso*-pentylaminoquinoline.



Plasmoquine.

Although originally derived from methylene blue, the constitutional formula of plasmoquine thus has analogies to that of quinine.

Observations in bird malaria showed that the chemotherapeutic index is 1 : 33 as compared with 1 : 4 for quinine (Roehl, 1926). Sioli (1926) and Mühlens (1926) found that plasmoquine is effective in human malarial infections due to benign tertian and quartan parasites, since it acts especially on the gametocytes of both species and on the trophozoites of *P. malariæ*, and to a rather less extent on those of *P. vivax*. In malignant tertian infections, however, the action on the schizonts is slight and temporary though, in compensation, plasmoquine possesses the remarkable property of destroying the gametocytes. The devitalising action is said to become apparent two days after the administration of the first dose. These observations were rapidly confirmed, but, owing to its failure in malignant tertian infections, plasmoquine was later given in association with quinine, the combination then proving effective in all forms of malaria. Swellengrebel and De Buck (1932) nevertheless observed that certain strains appear to be resistant to plasmoquine, since it was noted that, while in one district only a single relapse occurred among 66 patients with benign tertian infection treated with 0·03 gm. of plasmoquine and 15 gr. of quinine, yet in another district the same treatment was followed by about 50 per cent. of relapses (Malaria Commission of Holland, 1932). At first in treatment daily doses of plasmoquine simplex as large as 0·10 to 0·15 gm. were given and toxic reactions were by no means infrequent. Daily doses of 0·04 to 0·06 gm., or even of 0·02 gm., if prolonged for ten days or more (Missiroli and Marino, 1934), may produce similar toxic symptoms. In fact plasmoquine itself appears to have no advantages in the treatment of an acute attack of any form of malaria. For therapeutic purposes the Fourth General Report of the League of Nations Malaria Commission (1937) recommends for adults a daily dose of 0·01 to 0·03 gm., associated with quinine or atebrin, given either simultaneously or after a certain lapse of time. Even with such small doses, and especially when administered simultaneously with atebrin, toxic reactions may occur. Bastianelli, Mosna and Canalis (1937) found that 0·02 gm. continued for five days produced obvious ill effects such as asthenia, cyanosis, vomiting and gastralgia in nearly 50 per cent. of persons. Cyanosis is specially seen in the lips, tongue and, occasionally, in the lobules of the ears and in the finger nails. It is caused by the formation of methæmoglobin. Dixon (1933) noted that cyanosis is more likely to occur in patients suffering from chronic constipation. Tate and Vincent (1934) found that *in vitro* plasmoquine mixed with blood in a dilution of 1 in 50,000 is capable of producing methæmoglobin. Occasionally grave anæmia may be associated with the excretion of methæmoglobin in the urine. Other symptoms are dizziness, drowsiness, headache, fever, generalised urticaria, trembling (*cf.* Paladino-Blandini and Marino-Assereto, 1934) and occasionally icterus. According to Nocht and Mayer (1936), about six fatal cases have occurred. In one case, recorded by Blackie (1935), death was preceded by a gripping sensation in the throat and tightness of breath. The kidneys showed evidence of acute hæmorrhagic nephritis, the liver of parenchymatous necrosis. Similar changes can be produced experimentally in rhesus monkeys by the daily administration of from 0·0005 to 0·001 gm. per kilogram of body weight. The presence of a toxic liver necrosis is of some interest in view of the association of the quinoline nucleus with other compounds, such as cinchophen, which produce liver necrosis.

When injected intravenously in monkeys as an 0·1 per cent. solution in physiological saline, de Langen and Storm (1934) reported that plasmoquine causes a fall in blood pressure, while Eichholtz (1935) noted cardiac arrhythmia.

Nandi and Dikshit (1938) have reported a new test for determining plasmoquine in blood and tissues; in the urine some interfering substance appears to be present. A few drops of Folin's phenol reagent are added to an aqueous solution of plasmoquine and the mixture is made strongly alkaline by the addition of sodium carbonate. A blue colour develops slowly, reaching a maximum in about thirty minutes. Plasmoquine can be detected in a dilution of 1 in 200,000 by a Duboscq colorimeter or up to 1 in 1,000,000 by means of a spectrophotometer.

The question whether plasmoquine is liable to induce blackwater fever is as yet unsettled. The observations of Amy and Boyd (1936) in India suggest that in certain regions it may be responsible for an increase in the number of patients with hæmoglobinuria. It is possible also that prolonged use of plasmoquine may interfere with the new formation of hæmoglobin.

In so far as clinical prophylaxis is concerned there is now general agreement that it is essential to give very small doses of plasmoquine, not exceeding 0·01 gm. per diem, or, alternatively 0·02 gm. twice or even once a week (League of Nations Malaria

Commission, Fourth Report, 1937). In individuals, however, Rice (1932) and Brown (1933) have shown that daily doses of 0·03 to 0·06 gm. of plasmoquine may act as a true causal prophylactic : such doses, nevertheless, are verging on the toxic. In the Sudan, Henderson (1934) succeeded in reducing the morbidity of a population by two-thirds by a daily dose of 0·02 gm. plasmoquine, thus confirming the prophylactic results obtained by James, Nicol and Shute (1931) in induced malaria. However, for simple clinical mass prophylaxis it is doubtful whether plasmoquine is of greater value than either atebrin or quinine and plasmoquine, nor is there at present sufficient evidence to show its effect on the state of the spleens in malarial communities.

In pregnancy a combination of plasmoquine and quinine appears, according to Dikshit (1937), to be preferable to the administration of one of these drugs alone. In concentrations of 1 in 500,000 or 1 in 300,000, plasmoquine contracts the isolated uterus of the cat, dog and guinea-pig : in higher concentrations such as 1 in 100,000, it relaxes the uterine muscle.

The action of plasmoquine on malarial parasites appears to be direct, since morphological changes are readily seen microscopically in the gametocytes of *P. falciparum* as well as in those of *P. cathemerium* in birds (Wampler, 1930).

Oesterlin (1937), however, has brought forward evidence that substances, such as glucose, which play a part in cell respiration, increase the action of plasmoquine ; on the other hand the action of quinine is unaltered by the addition of such respiratory metabolic products.

References

AMY, A. C., and BOYD, J. S. K., 1936, Malaria in India : the synthetic drugs and the relapse rate. *J. R. Army med. Corps.*, LXVII., 83.

BASTIANELLI, G., MOSNA, E., and CANALIS, A., 1937, Prevention and treatment of malaria by synthetic drugs. *Bull. Hlth. Organ, League of Nations*, VI., 822.

BLACKIE, W. K., 1935, A fatal case of plasmoquine poisoning. *S. Afr. med. J.*, IX., 147.

BROWN, J. Y., 1933, The care, inspection and transportation of live anopheles for use in the malaria therapy of general paralysis. *W. Afr. med. J.*, VII., 78.

De Langen, C. D., and Storm, C. J., 1934, Experimenteel onderzoek van circulatiestoornissen door plasmochine en atebrine. *Geneesk. Tijdschr. Ned.-Ind.*, LXXIV., 1646.

Dikshit, B. B., 1937, A preliminary note on the action of plasmoquine in pregnancy. *Rec. Mal. Surv. India*, VII., 77.

Dixon, H. B. F., 1933, A report on 600 cases of malaria treated with plasmoquine and quinine. *J. R. Army med. Corps*, XL., 431.

Eichholtz, F., 1935, " Experimentelle Untersuchung von Zirkulationsstörungen durch Plasmochin und Atebrin." Bemerkungen zur Arbeit von de Langen uhd Storm. *Klin. Wschr.*, XIV., 714.

Guttman, P., and Ehrlich, P., 1891, Über die Wirkung des Methylenblau bei Malaria. *Berl. klin. Wschr.*, XXVIII., 593.

Henderson, L. H., 1934, Prophylaxis of malaria in the Sudan with special reference to the use of plasmoquine. *Trans. R. Soc. trop. Med. Hyg.*, XXVII., 285.

Hörlein, H., 1926, Ueber die chemischen Grundlagen und die Entwicklungsgeschichte des Plasmochins. *Beih. Arch. Schiffs- u. Tropenhyg.*, XXX., 305.

James, S. P., Nicol, W. D., and Shute, P. G., 1931, On the prevention of malaria with plasmoquine. *Lancet, ii.*, 341.

Malaria-Commissie uit den Gezondheidsraad, 1932, Jaarverslag over de Jaren 1930 en 1931 van de Malaria-Commissie uit den Gezondheidsraad. *Versl. Volksgez.*, July.

Missiroli, A., and Marino, P., 1934, Anwendung des Chinoplasmin zur Malariasanierung. *Arch. Schiffs- u. Tropenhyg.*, XXXVIII., 1.

Mühlens, P., 1926, Die Behandlung der natürlichen menschlichen Malariainfektionen mit Plasmochin. *Beih. Arch. Schiffs- u. Tropenhyg.*, XXX.. 325.

Nandi, B. K., and Dikshit, B. B., 1938, A new colorimetric test for plasmoquine. *Indian J. med. Res.*, XXV., 937.

Nocht, B., and Mayer, M., 1936, " Die Malaria," 2nd Edit. Berlin.

Oesterlin, M., 1937, Studien zur Chemotherapie der Malaria. *Arch. Schiffs- u. Tropenhyg.*, XLI., 720.

Paladino-Blandini, A., and Marino-Assereto, P., 1934, La chinoplasmina nella profilassi della malaria a Schiavonea (bonifica di Sibari). *Riv. Malariol.*, XIII., 161.

Rice, J. B., 1932, Experiments on plasmoquine as a prophylactic among West African negroes exposed to bites of *A. costalis*. *Ann. trop. Med. Parasit.*, XXVI., 555.

Roehl, W., 1926, Die Wirkung des Plasmochins auf die Vogelmalaria. *Beih. Arch. Schiffs- u. Tropenhyg.*, XXX., 311.

Schulemann, W., 1932, Synthetic anti-malarial preparations. *Proc. R. soc. Med.*, XXV., 897.

Schulemann, W., Schönhöfer, F., and Wingler, A., 1932, Synthese des Plasmochin. *Klin. Wschr.*, XI., 381.

Sioli, F., 1926, Prüfung des Plasmochins bei der Impfmalaria der Paralytiker. *Beih. Arch. Schiffs- u. Tropenhyg.*, XXX., 319.

Swellengrebel, N. H., and De Buck, A., 1932, Plasmoquine prophylaxis in benign tertian malaria. *Proc. R. Acad. Sci. Amsterdam*, XXXV., 911.

Tate, P., and Vincent, M., 1934, The action of atebrin on bird malaria. *Parasitology*, XXVI., 523.

Wampler, F. J., 1930, A preliminary report on the early effects of plasmochin on *P. cathemerium*. *Arch. Protistenk.*, LXIX., 1.

HOMOLOGUES OF PLASMOQUINE

The characteristic feature of the synthetic anti-malarial drugs is the presence of a dialkylaminoalkylamino side chain: $HN(CH_2)_n.NRR'$. In both plasmoquine and atebrin the chain is branched $n = 5$, $HN.CH(CH_3)CH_2.CH_2.CH_2.N(C_2H_5)_2$, and is attached to a methoxyquinoline and a chloromethoxyacridine nucleus respectively. As a result a number of quinoline and acridine derivatives have been prepared and tested for their anti-malarial action. Thirteen synthetic quinoline derivatives (*cf.* Barger and Robinson, 1929) were tested by Tate and Vincent (1933) for their action in curing bird malaria. The chemotherapeutic indices of two, the compounds 8-γ-aminopropylamino-6-ethoxyquinoline (R = 25), and the corresponding methoxyquinoline (R = 36) were 1 : 16, as compared with 1 : 30 for plasmoquine. The action of these compounds closely resembled that of plasmoquine, while 8-γ-aminopropylamino-6-n-butoxyquinoline had a sterilising action on *Plasmodium relictum* equal to, if not greater than, that of plasmoquine, although its chemotherapeutic action was only 1 : 8. R 36 was found, however, to be quite inactive in human infections due to *P. falciparum* and was also liable to produce methæmoglobinæmia.

Of the homologues of plasmoquine that have been tested clinically the majority are 6-methoxyquinolines with a dialkyl-amino-alkylamino side chain in position 8 and, as pointed out by Henry and Gray (1935), it is the length and nature of this side chain which is the principal source of variation. Thus of forty-eight compounds of this character prepared by Fourneau and his colleagues (1931 and 1933) no less than thirty-eight possessed curative action in bird malaria. The majority, however, were highly unstable and soon became toxic. The compounds, however, in the following table, in which the character of the side chain is shown, were found to have low toxicity combined with high curative action.

According to Fourneau and his colleagues these compounds are considerably less toxic than plasmoquine for the rabbit, and in Java sparrows infected with *Hœmoproteus paddœ* have chemo-

Compound.	Name of Drug.	Character of Side Chain.
6-methoxy-8-diethylamino-*iso*-pentylaminoquinoline	Plasmoquine	$NH.CH(CH_3).CH_2.CH_2.CH_2.N(C_2H_5)_2$
6-methoxy-8-dimethylamino-*n*-propylaminoquinoline	Fourneau 574	$NH.CH_2.CH_2.CH_2.N(CH_3)_2$
6-methoxy-8-diethyl-aminoamyl-aminoquinoline	Fourneau 664	$NH.CH_2.C(CH_3)_2.CH_2.N(C_2H_5)_2$
6-methoxy-8-diethylamino-*n*-propylaminoquinoline	Fourneau 710 (Rhodoquine)	$NH.CH_2.CH_2.CH_2.N(C_2H_5)_2$
8-diethylamino-*n*-propylamino-quinoline	Fourneau 728	$NH.CH_2.CH_2.CH_2.N(C_2H_5)_2$
6-methyoxy-8-diethylamino-*n*-amylaminoquinoline	Fourneau 735	$NH.CH_2.CH_2.CH_2.CH_2.CH_2.N(C_2H_5)_2$
8-diethylamino-undecylamino-6-methoxyquinoline	Fourneau 852	$NH.(CH_2)_{11}N(C_2H_5)_2$

therapeutic indices superior to that of plasmoquine (C.I. = 1 : 40) : No. 710 (Rhodoquine *) has a chemotherapeutic index of 1 : 100 ; No. 735, 1 : 150 ; No. 664, 1 : 40. As a result of their observations they believe that the methoxy group in position 6 in the quinoline nucleus is not essential for anti-malarial action. On the other hand, the substitution of $—OCH_3—$ for $—OC_2H_5—$ appears always to exert an unfavourable action, as does the group $—C.CH_2—O—R$ placed in the diamino chain. In at least one compound replacement of dimethylamino by diethylamine reduced toxicity some ten times.

Fourneau 574 was found to be less toxic than 710 and very stable in a tropical climate by Massias (1933), who treated 43 patients with this drug combined with 1 gm. of quiniostovarsol. Only two patients, who vomited, showed toxic symptoms, while parasites were not found later than seven days after the beginning of treatment. When given alone (Massias, 1934 [1 & 2]), however, early relapses occurred both in benign and malignant tertian infections.

Fourneau 664 has been tested in human infections due to *P. vivax* by Monier (1931[1]), who found that while daily doses of 0·04 gm. were insufficient to remove parasites from the blood, 0·1 gm. produced toxic symptoms. Doses of 0·06 to 0·08 gm. daily, injected intramuscularly as a 1 per cent. solution, gave good results and all parasites were removed with total doses of

---

* The term "rhodoquine" was originally applied to all the oxyquinolines prepared by Fourneau and his colleagues but is now restricted to 710.

0·3 gm. Relapses were prevented by the administration of 0·5 gm. Fourneau 710 (rhodoquine) is a yellow powder freely soluble in water. The Russian product, plasmocide, is described as being chemically identical with Fourneau 710. Its action in bird malaria on the sporozoites of *P. relictum* is said to be similar to that of plasmoquine. It appears to have certain of the defects of plasmoquine and to be somewhat less effective as regards its action on benign tertian and quartan parasites, the effect of 0·06 gm. of plasmocide corresponding therapeutically with that of 0·02 gm. of plasmoquine, but it is somewhat more easily prepared. Tarejev (1935), who treated 695 malarial patients in Moscow with plasmocide, observed toxic reactions, chiefly epigastric pains, vomiting and diarrhœa, in 7 per cent. With prophylactic doses at fairly long intervals no serious reactions have been reported. Cerebellar ataxia, polyneuritis and partial optic atrophy have, however, been recorded. Fourneau 710 was used by Monier (1931[2]) with good results in human infections due to *P. vivax*. Sautet (1932), in Corsica, found this compound of little value, but in combination with quinine acetarsol it was active in all three forms of malaria. Sergent, Sergent, Trensz and Vogt (1932), in Algeria, obtained similar results from the use of 710 alone; vomiting and nausea were not uncommon. According to Sicault and Decourt (1934) daily doses of 0·018 to 0·048 gm. (0·3 to 0·8 mgm. per kilogram of body weight) caused the rapid disappearance of all parasites except the schizonts of *P. falciparum*. No toxic symptoms were noted, but larger doses of from 3·6 to 10 mgm. per kilogram of body weight proved toxic. With non-toxic doses relapses were not prevented. Berny and Nicolas (1936) gave 0·02 gm. of Fourneau 710 combined with 0·3 gm. of atebrin (quinacrine) once a week for six months as a prophylactic and found no sign of intolerance to the drugs. Fourneau 852 is said to be much less toxic than plasmoquine and to be effective against all forms of malarial parasites. Bovet and Demanche (1933) found that it acted as powerfully as atebrin in bird malaria due to *P. relictum* and in infections due to *Hæmoproteus paddæ* in the Java sparrow. In the treatment of human malarial infections Fourneau 852 has been combined with sodium acetarsol. This compound, Fourneau 915 or rhodoquine V, has been tested by Sergent and

Vogt (1933), who found that 0·6 gm. daily caused complete disappearance of all forms of parasites within five days: relapses were, however, not infrequent while occasionally vomiting occurred. With daily doses of 0·9 gm. severe toxic symptoms developed.

The action of a series of homologues of plasmoquine with unbranched chains, where $n = 2$ to 11, has been studied by Bovet, Benoit and Altman (1934). Hæmoproteus infections in the Java sparrow and avian malaria in canaries were investigated. For hæmoproteus infections the graph obtained by plotting minimal effective doses of the various compounds against values of n is roughly parabolic with summits at $n = 3$ and $n = 5$: it may be taken as a measure of activity against the sexual forms of the parasites. When, however, the action of the same set of compounds is tested on avian malaria in canaries the anti-malarial activities for the higher values of n are almost as great as for the lower values of n in the Java sparrow. Thus when $n = 11$ the chemotherapeutic index is 1 : 100 for the canary and 1 : 3 for the Java sparrow, and it is assumed that the higher activity shown in the canary must be due to action on both the sexual and asexual forms of the parasites. If this assumption is correct any increase of molecular complexity, as in the replacement of quinoline by acridine when passing from quinoline to atebrin or by lengthening the side chain in plasmoquine, must cause a change from activity against sexual to activity against asexual forms. There thus seems no reason, at any rate theoretically, why, by synthesis, compounds should not be prepared with high activity both against the sexual and asexual forms of malaria parasites.

Under the name of "Certuna" a dialkylamino-butylamino-hydroxyquinoline has been studied in bird malaria by Kikuth (1938) and by Mühlens (1938) and Sioli (1938) in natural and induced infections in man. Its action is mainly on the male gametocytes.

References

Barger, G., and Robinson, R., 1929, Attempts to find new anti-malarials. *J. chem. Soc.*, 2947.

Berny, P., and Nicolas, L., 1936, Note sur la campagne antipaludique effectuée en 1936 à la Crique Anguille (Guyane française) avec la médication mixte (quinacrine-rhodoquine). *Bull. Soc. Path. exot.*, **XXIX.**, 870.

BOVET, D., BENOIT, G., and ALTMAN, R., 1934, Action thérapeutique de quinoléines à poids moléculaire élevé, homologues de la plasmoquine, sur les hématozoaires des calfats et des serins. *Ibid.*, **XXVII.**, 236.

BOVET, D., and DEMANCHE, L., 1933, Nouveaux produits actifs dans le paludisme aviaire : une quinoléine de synthèse agissant sur les schizontes et sur les gamètes (F. 852). *Ann. Inst. Pasteur*, **LI.**, 528.

FOURNEAU, E., TRÉFOUEL, J., TRÉFOUEL, Madame, BOVET, D., and BENOIT, G., 1931, Contribution à la chimiothérapie du paludisme. Essais sur les calfats. *Ibid.*, **XLVI.**, 514.

FOURNEAU, E., 1933, Contribution à la chimiothérapie du paludisme. Essais sur les calfats (deuxiéme mémoire). *Ibid.*, **L.**, 731.

HENRY, T. A., and GRAY, W. H., 1935, Trypanocidal and anti-malarial drugs. *Trop. Dis. Bull.*, **XXXII.**, 385.

KIKUTH, W., 1938, Zur weiterentwicklung der Chemotherapie der Malaria. "Certuna," ein neues Gametenmittel. *Klin. Wschr.*, **XVII.**, 524.

MASSIAS, C., 1933, Le "574" associé au quino-stovarsol dans le traitement du paludisme à *Plasmodium vivax* et à *Plasmodium præcox*. *Bull. Soc. Path. exot.*, **XXVI.**, 590.

MASSIAS, C., 1934 [1], Nouvelles observations de 48 paludéens traités par un dérivé quinoléique associé au quiniostovarsol. *Ibid.*, **XXVII.**, 641.

MASSIAS, C., 1934 [2], Dérivé quinoléinique employé seul contre le paludisme à *P. vivax* et à *Pl. præcox*. *Ibid.*, **XXVII.**, 644.

MONIER, H. M., 1931 [1], Essai d'un dérivé de la quinoléine (664 Fourneau) dans la malaria expérimentale. *Ibid.*, **XXIV.**, 93.

MONIER, H. M., 1931 [2], Essais thérapeutiques du 710 Fourneau dans quelques cas de paludisme. *Ibid.*, **XXIV.**, 97.

MÜHLENS, P., 1938, "Certuna," ein neues Tropikagametenmittel. *Dtsch. med. Wschr.*, **LXIV.**, 295.

SAUTET, J., 1932, Traitement du paludisme par le 710 et le 574 associés ou non à la quinine ou au stovarsol. *Bull. Soc. Path. exot.*, **XXV.**, 1074.

SERGENT, E., SERGENT, A., TRENSZ, F., and VOGT, D., 1932, Essais de traitement du paludisme à *Plasmodium præcox* par le 710 Fourneau seul ou associé à la quinine chez les paludéens d'Algérie. *Arch. Inst. Pasteur, Algérie*, **X.**, 1.

SERGENT, A., and VOGT, P., 1933, Essais de traitement de la tierce maligne par le "915 Fourneau" ou rhodoquine. *Bull. Soc. Path. exot.*, **XXVI.**, 1255.

SICAULT, G., and DECOURT, P., 1934, Observations sur 25 paludéens traités par la rhodoquine (710 F.). *Ibid.*, **XXVII.**, 146.

SIOLI, F., 1938, Prüfung des neuen Malariamittels "Certuna" bei der Impfmalaria der Paralytiker. *Klin. Wschr.*, **XVII.**, 527.

TAREJEV, E., 1935, Efficacité des dérivés synthétiques antipaludéens fabriqués en U.R.S.S. Note dactylographiée (quoted in Fourth General Rep. Malaria Commission, *League of Nations*, *Bull. of the Health Organisation*, 1937, **VI.**, 917).

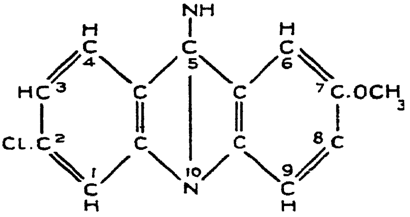
TATE, P., and VINCENT, M., 1933, The action of synthetic quinoline compounds on avian malaria. *Parasitology*, **XXV.**, 411.

ATEBRIN

The success which attended the insertion of a dialkylaminoalkylamino side chain in 6-methoxyquinoline, naturally led to an examination of the results of such insertions in other heterocylic nuclei. Eventually the compound, first termed erion, plasmoquine

E and eventually atebrin, or in the United States of America atabrine, was produced. According to Mauss and Mietzsch (1933), atebrin is 2-chloro-5-diethylamino-*iso*-pentylamino-7-methoxy-acridine dihydrochloride. It thus contains the same side chain as plasmoquine, with the 6-methoxyquinoline nucleus of the latter replaced by a 2-chloro-7-methoxyacridine nucleus.

$$CH(CH_3).CH_2.CH_2.CH_2.N.(C_2H_5)_2,\ 2HCl.$$



Atebrin.

Quinacrine, a French product, has the same constitutional chemical formula, while in the Union of Soviet Socialist Republics the name " acriquine" has been given to a similar compound. [Rubinstein (1936), however, states that the designation " acriquine " has been applied to the dihydrochloride of 2-methoxy-6-chloro-9-diethylamino-butylaminoacridine when exposed to ultra-violet light.] " Atebrin " is a yellow powder with a somewhat bitter taste, soluble to the extent of approximately 2 or 3 per cent. at room temperature or 7 per cent. in water at 40° C. Like quinine, atebrin is fluorescent. Christophers (1937) states that the base is soluble in water to the extent of $10^{-4 \cdot 08}$ (0·000084), the undissociated base to the extent of $10^{-4 \cdot 8}$ (0·000016). The solubility product is $10^{-8 \cdot 68}$. As was pointed out by Magidson and Grigorowsky (1936), watery solutions of atebrin will not keep indefinitely, but decompose by slow hydrolysis with the formation of the sparingly soluble 2-methoxy-6-chloro-acridone and the very soluble α-diethylamino-δ-aminopentane dihydrochloride.

Mietzsch, Mauss and Hecht (1936) found that a 1 per cent. solution of atebrin dihydrochloride could be kept for three weeks at a temperature of 37° C. without any formation of 2-methoxy-6-chloro-acridone, but after heating for a quarter of an hour at

105° C. decomposition began after a few days. Neither of the decomposition products showed any toxic action in animals.

For parenteral administration atebrin dimethanesulphonate has been prepared, two molecules of methanesulphonic acid (CH_3SO_3H) replacing two molecules of hydrochloric acid in the dihydrochloride. This salt "atebrin-musonate" is extremely soluble in water, but even here it is preferable to employ only freshly prepared solutions and not to submit these to prolonged boiling.

While atebrin dihydrochloride and the base can be readily obtained, the monohydrochloride cannot be obtained in an isolated form, owing to the low solubility of the base and the close proximity of the two dissociation constants ($pk_2 - pk_1 = 2{\cdot}59$). The presence of the salt, owing to its bright red colour, can however be detected both in the solid form and in solution.

According to Christophers (1937) atebrin possesses two dissociation constants, both probably connected with the amine portion of the molecule, one very strong, $pk_1 = 3{\cdot}88$, the other weaker, $pk_2 = 6{\cdot}47$. There is some indication of a third constant, probably in relation to the acridine ring, but if a third constant exists it is weaker than $pk_3 = 11{\cdot}0$.

Kikuth (1932) was the first to report on the effects on bird malaria. When tests were made on canaries infected with *Plasmodium præcox* and on Java sparrows infected with *Hæmoproteus oryzivoræ* atebrin destroyed the schizonts but differed from plasmoquine in not being a gametocide. Tests in induced and naturally occurring human malaria carried out by Sioli (1932), Peter (1932) and Mühlens (1932) showed that it had a similar action in man, since it resembles quinine in destroying all forms of benign tertian and quartan parasites and also the schizonts of malignant tertian, although the crescents are untouched.

The Pharmacology of Atebrin. Pharmacologically the action of atebrin is not especially noteworthy. Beyond a slight irritant action on the gastric mucosa there are no effects referable to the gastro-intestinal canal. Ganguli (1933) has noted in man an occasional slight lowering of blood pressure, while de Langen and Storm (1934 1) and Storm (1935) have recorded a fall in both diastolic and systolic blood pressure as a result of the intravenous injection of atebrin in monkeys. This effect appears to be chiefly due to action on the vasomotor centres. Hecht (1935) has obtained similar effects in cats, following the intravenous injection of 2 mgm. per kilogram of body weight. Intramuscular injections of 20 mgm. per kilogram of body weight are, however, without effect on the blood pressure of cats. In man intravenous injections of 0·2 gm. of atebrin are without effect on the blood pressure, while any fall in pressure can be counteracted by the injection of adrenalin (Storm, 1935). In any case there is little need to administer atebrin intravenously. According to Chin (1937) the excised heart of the rabbit is rapidly paralysed by direct toxic action on the heart muscle. De Langen and Storm (1934^2) found that in normal healthy individuals atebrin does not produce urobilinuria, but it may do so in persons whose liver is poor in glycogen as a result of malnutrition. Small doses of atebrin stimulate both the pregnant and non-pregnant uterus in the rabbit and guinea-pig. Large doses stimulate and later paralyse the uterus. In nerve muscle preparations atebrin depresses the excitability of motor nerve endings (Chin, 1937). Unlike quinine, which disappears quickly, atebrin, though it cannot be detected in the blood for more than twenty-four hours after injection (Chopra, Ganguli and Roy, 1936), remains in the body for considerable periods. Thonnard-Neumann and Le Doux (1931) detected its presence in the urine thirty-six days after the last dose ; Jarvis (1932) recorded its presence on an average twenty-six days after the conclusion of treatment : in one patient it appeared on the second day of treatment and was present for thirty-seven days. Massa (1933) believes that by examining the urine with either a Wood light or Wood filter fluorescence can be detected for sixty or seventy days after the cessation of treatment. Although, as shown by Tropp and Weise (1933), from 50 to 70 per cent. of atebrin is excreted in the urine, according to Kehar ($1935^{1 \& 2}$) a considerable proportion of the drug is assimilated, transformed and destroyed, after temporary accumulation, in the organism, and hence finally excreted in the form of disintegration products of the original molecule. The quantity of atebrin excreted by the bowel has not yet been accurately determined. Analysis by fluorescence appears to prove conclusively the contention of Foy, Kondi and Peristeris (1936) that part of the atebrin absorbed is accumulated in the cells of the liver and spleen, while clinical proof of the partial accumulation of atebrin is afforded by the staining of the histiocytes of the skin. The quantity retained depends, however, on a number of factors. Absorption, for instance, depends on the presence or absence of food in the stomach, hypoacidity of the stomach or inflammation of the intestinal mucosa. Chopra, Sen and Roy (1937) found that atebrin in combination with plasmoquine in dragées was not absorbed, while atebrin in tablet form by itself was readily absorbed. The intervals at which the drug is administered influence the amount retained, while small doses are more rapidly eliminated than large doses. In this connection the results reported from Malaya by Field, Niven and Hodgkin to the Fourth Report of the Malaria Commission of the League of Nations (1937) are of interest, since they show the excretion of atebrin in the urine after the administration of 0·4 gm. of atebrin weekly for one year.

EXCRETION OF ATEBRIN AFTER CESSATION OF PROPHYLACTIC TREATMENT.

(Field, Niven and Hodgkin, 1937.)

Fluorescence Due to Atebrin in the Urine.

Period since cessation of prophylactic treatment.	*No. of samples examined.*	*Strong.*	*Moderate.*	*Weak.*	*Absent.*
1 week . .	50	17	23	10	0
2 weeks . .	47	4	24	16	3
3 „ . .	46	2	7	26	11
4 „ . .	41	0	2	26	13
5 „ . .	41	1	0	3	37
6 „ . .	48	0	0	3	45
7 „ . .	20	0	0	0	20

Estimation of Atebrin in the Blood, Tissues and Urine. Various methods for the quantitative determination of atebrin in the urine have been reported by Green (1932), Tropp and Weise (1933), Hecht (1933), Wats and Ghosh (1934), Hicks (1935), Schechter and Taylor (1936) and Weise (1937). In general these methods consist of extraction of the urine with an immiscible solvent, evaporation of the solvent and solution of the residue in an acid, or extraction from the solvent by an acid and comparison of the colour or the fluorescence of the resulting solution with appropriate standards. Schechter and Taylor (1936) have substituted sulphuric acid for hydrochloric acid as used by Tropp and Weise (1933), thus avoiding the possibility of confusion from the presence in the urine of quinine, quinidine or even plasmoquine, while Weise (1937) objects to the use of amyl alcohol for extraction of atebrin from the urine, since other pigments, such as urobilin, may also be extracted. A method applicable to the estimation of atebrin in the blood, urine or tissues has been reported by Hecht (1936), who found that the procedure adopted by Chopra and Roy (1935 and 1936) was unsatisfactory. After addition of an equal quantity of 60 per cent. caustic potash to the urine, blood and tissues and heating on a water bath for five minutes, an amount of distilled water is added equal to that of the caustic soda. The homogeneous fluid mass is then extracted with 8 parts of benzene and 2 parts of amyl alcohol (2 c.c. per 1 gm. of tissue). After centrifugation, 75 per cent. of the volume of the solution is removed and shaken with a measured amount of N/1 HCl. After the solution has settled, 1 c.c. of the hydrochloric acid layer is removed and the solution is alkalinised and shaken with a small quantity of amyl alcohol. The colour of the amyl alcohol solution is then compared with that of a standard atebrin solution similarly treated. Chopra and Roy (1937) prefer to digest the tissues with papain, but the whole method has been criticised by Gentzkow (1938) on the ground that it is inaccurate when the amount of atebrin to be estimated is small and the recommended amount of alkali is used.

The following method, described by Gentzkow (1938), has been found accurate to measure differences of 0·1 mgm. per litre. To 45 c.c. of acetone are measured 5 c.c. of oxalated blood with constant shaking. Shaking is continued for a few minutes and then 2 c.c. of a 20 per cent. aqueous solution of neutral lead acetate are added, thoroughly mixed and the mixture transferred to a 50 c.c. centrifuge tube, which is centrifuged at high speed for five minutes. The precipitated proteins are separated from the clear supernatant liquid to which are added 5 c.c. of water. The mixture is evaporated to about 2 c.c. on a water bath, cooled and added to 0·5 c.c. of 30 per cent. phosphoric acid and made up to the 5 c.c. mark with water. This mixture is then filtered through a No. 42 Whatman filter paper. Of the filtrate 4 c.c. is transferred to a small test tube, and for each cubic centimetre 0·1 c.c. of modified Tanret's reagent is added. The mixture is heated on a boiling water bath for five minutes, then cooled. This treatment produces a very fine precipitate which remains suspended for a considerable time.

The Toxicity of Atebrin. Occasionally in adults and perhaps more commonly in children, symptoms of immediate intolerance such as regurgitation and vomiting may be noted. Apart from the yellow discoloration of the skin, which is seen in patients who have been taking atebrin, the main toxic symptoms are referable to the central nervous system and the gastro-intestinal tract. This is in accordance with the experimental findings of Hecht (1933), who observed that in animals given large doses gastro-intestinal and cerebral irritation resulted.

The gastro-intestinal symptoms consist of abdominal pains, nausea, vomiting and rarely diarrhœa. The nervous symptoms comprise headache, occasionally severe, insomnia, temporary giddiness and faintness, delirium and epileptiform attacks.

Kingsbury (1934) observed psychoses in twelve patients. In eight the symptoms continued for from twelve hours to seven days, the average duration being thirty-six hours, but in four others the mental derangement was so severe that removal to mental hospitals was necessary. During the Ceylon epidemic, cases of delirium occurred after both the oral administration of atebrin dihydrochloride and intramuscular injection of atebrin musonate (*cf.* Briercliffe, 1935; Udalagama, 1935; and Hay, Spaar and Ludovici, 1935).

Epileptiform fits were noted in Singapore by Vardy (1935) after two injections of 0·375 gm. of atebrin musonate, and by van Slype (1936) who, with doses of 0·1 gm., observed three cases of convulsions in children under eighteen months : one child died. Van Heukelom and Overbeek (1936) also reported epileptiform fits with death in two patients. Organic cerebral lesions were found in both cases.

Collapse and death has also been recorded. Briercliffe (1935) believes that in the Ceylon epidemic 0·5 per cent. of the hospital patients treated with atebrin musonate died as a result of the drug.

In a case reported by Fernando and Wijerama (1935), in which death occurred two months after two intramuscular injections, atebrin was found in the pericardial and pleural fluid. It is still uncertain whether after long continued administration atebrin may exert a toxic action on the liver. It would seem that personal idiosyncrasy plays a large part in the occurrence of toxic symptoms after atebrin, for instances have been reported where, by accident, in Spain relatively enormous doses have been given. Barbosa (1934), for instance, gave several patients, among them children, ten times the normal dose, while in Greece, Foy, Kondi and Peristeris (1936) observed patients who had taken ten and fifteen tablets at the same time.

The symptoms of poisoning were very slight and consisted mainly of nausea and vomiting.

The yellow pigmentation, so commonly present as a result of the administration of atebrin, is most clearly seen on the dorsum of the hands and feet and on the forehead and face, but not on the scleræ. Occasionally it may persist for as long as eighteen weeks after cessation of treatment (Schechter and Taylor, 1936). Various predisposing causes have been suggested, such as a hot, dry climate leading to heavy sweating, anæmia and defective functioning of the liver and kidneys.

ATEBRIN IN THE TREATMENT OF HUMAN MALARIA

As a result of the experience gained during the past few years there is now general agreement that for adults the daily dose of atebrin should be 0·3 gm. This dose has a slightly more rapid action on the trophozoites of *Plasmodium vivax* than quinine in doses of 1 gm. In addition, the parasiticidal action lasts longer after the end of treatment with atebrin than with quinine. On the trophozoites of *P. malariæ* the action of atebrin is of the same nature, while in 90 per cent. of cases the trophozoites of *P. falciparum* disappear from the peripheral blood after the fourth dose of atebrin. Atebrin thus, as a rule, acts more swiftly than quinine, but differences between strains of parasite prevent the drawing of uniform conclusions. The action of atebrin on the gametocytes of all species is similar in nature to that of quinine, but perhaps slightly more efficient as regards those of *P. vivax* and *P. malariæ*.

In children relatively larger doses of atebrin are necessary to obtain the same results as in adults, and, in addition, children are more sensitive to the toxic action of the drug. De (1937) gave 0·1 gm. of atebrin musonate dissolved in 3 c.c. of distilled water on two successive days to children aged from six months to six years: the injections were made into the buttock.

The action of atebrin in controlling the clinical symptoms of benign and malignant tertian infections is very rapid, and in some endemic regions is even more energetic in malignant tertian than in benign tertian infections. In other cases the contrary seems to be true. In benign tertian infections, fever nearly always falls after the first three therapeutic doses, after, that is to say, the second febrile paroxysm. Injections of atebrin musonate control the clinical symptoms even more quickly than atebrin and remove asexual forms of the parasites from the blood. Crescents are neither destroyed nor is their formation prevented. The number of relapses is somewhat less than after quinine, especially in the case of benign tertian and of some strains of malignant tertian parasites. In communities treated with atebrin the spleen rate appears to decrease perhaps somewhat more slowly than with quinine. Bastianelli, Mosna and Canalis (1937), for instance, in Sardinia noted little or no reduction in the size of the spleen after doses of 0·1 to 0·3 gm. of atebrin. On the other hand, the effects of atebrin continue to produce an effect for a longer period after the cessation of treatment, the decrease in the percentage of enlarged spleens continues longer and the return of the splenic index figures to their former level occurs more slowly than when quinine is employed. The exact effect of atebrin on the process of immunisation requires further study, as do the toxic results of prolonged administration. Fischer (1936) quotes instances of patients who have taken daily therapeutic doses of atebrin for as long as two and a half years without toxic results.

The Prophylactic Action of Atebrin. There is at present some evidence, derived from the experiments of James (1932 and 1936), on certain strains of *P. vivax*, and Soesilo, Gilbert and Bangindo (1933) on *P. falciparum*, that atebrin, in large but harmless doses, may exert some degree of true causal prophylactic effect under certain conditions, but the results obtained by Ciuca *et al.* (1937) strongly support the view that this action is upon the schizogenic forms of the parasite in red blood corpuscles rather than upon the sporozoites. Ciuca and his colleagues were able to show that the sporozoites of *P. falciparum* in the salivary glands of anophelines are able to withstand a concentration of 1 in 2,500 atebrin for thirty minutes, since they are afterwards able to cause infection in susceptible human subjects.

So far as clinical and mass prophylaxis are concerned there is, however, evidence that bi-weekly doses of 0·2 gm. of atebrin administered during the whole of the malaria transmission season and even for a few weeks longer exerts a definite action on the number of malarial attacks (*cf.* Bispham, 1936, and Fourth Report of League of Nations Malaria Commission, 1937).

The Action of Atebrin on Malaria Parasites. The evidence, which however is not extensive, suggests that the action of atebrin on the trophozoites of malaria parasites is direct. James (1934) has shown that after a single dose of 0·6 gm. of atebrin, morphological changes rapidly occur in the parasites of benign tertian and quartan malaria. The pigment becomes aggregated into clumps and disappears, while the cytoplasm is ragged and also eventually disappears : the nuclear vacuole is distended, the chromatin opens up and becomes diffuse till only a few lightly stained dots remain. Similar changes have been noted by Mosna (1936) and by Chopra *et al.* (1936) as a result of examining the action of atebrin on *P. knowlesi.* Fischl and Singer (1935) have made the important observation that a number of fluorescent dyes, including atebrin, are fixed by the plasmodia of bird malaria.

References

Barbosa, A., 1934, Contribution au traitement du paludisme par l'atébrine. *Med. País. cálidos*, **VII.**, 73, 123 and 157.

Bastianelli, G., Mosna, E., and Canalis, A., 1937, Prevention and treatment of malaria by synthetic drugs. *Bull. Hlth. Organ. League of Nations*, **VI.**, 822.

Bispham, W. N., 1936, A report on the use of atabrine in the prophylaxis of malaria. *Amer. J. trop. Med.*, **XVI.**, 547.

Briercliffe, R., 1935, The Ceylon Malaria epidemic 1934–1935. Report by the Director of Medical and Sanitary Services. Ceylon Government Press : Colombo.

Chin, K., 1937, Ueber die pharmakologische Wirkung des Atebrins. *J. med. Ass. Formosa*, **XXXVI.**, 138.

Chopra, R. N., Das Gupta, B. M., and Roy, A. C., 1936, On the mode of action of atebrin on *Plasmodium knowlesi*. *Indian med. Gaz.*, **LXXI.**, 710.

Chopra, R. N., Ganguli, S. K., and Roy, A. C., 1936, Studies on the action of anti-malarial remedies on monkey malaria. The relationship between the concentration of atebrin in the circulating blood and parasite count. *Ibid.*, **LXXI.**, 443.

Chopra, R. N., and Roy, A. C., 1935, On the estimation of minute quantities of atebrin in the blood. *Ibid.*, **LXX.**, 504.

Chopra, R. N., and Roy, A. C., 1936, On the determination of small quantities of atebrin in the blood. *Indian J. med. Res.*, **XXIV.**, 487.

Chopra, R. N, and Roy, A. C., 1937, On the estimation of atebrin in tissues. *Ibid.*, **XXV.**, 455.

Chopra, R. N., Sen, B., and Roy, A. C., 1937, Individual variations in the effectiveness of synthetic anti-malarial drugs (a preliminary note). *Indian med. Gaz.*, **LXXII.**, 131.

Christophers, S. R., 1937, Dissociation constants and solubilities of bases of anti-malarial compounds. I. Quinine. II. Atebrin. *Ann. trop. Med. Parasit.*, **XXXI.**, 43.

Ciuca, M., Ballif, L., Chelarescu, M., Isanos, M., and Glaser, L., 1937, Action of quinine and atebrin on the sporozoites of *Plasmodium falciparum*. *Trans. R. Soc. trop. Med. Hyg.*, **XXXI.**, 235.

De, R. K., 1937, Treatment of malaria in children with atebrin musonate. *Indian med. Gaz.*, **LXXII.**, 290.

De Langen, C. D., and Storm, C. J., 1934^{1}, Experimenteel onderzoek van circulatiestoornissen door plasmochin en atebrine. *Geneesk. Tijdschr. Ned.-Ind.*, **LXXIV.**, 74.

De Langen, C. D., and Storm, C. J., 1934^{2}, Observations on the modern medical treatment of malaria. A clinical and experimental study. *Trans. 9th Congr. Far Eastern Ass. trop. Med. Nanking*, **II.**, 233.

FERNANDO, P. B., and WIJERAMA, E. M., 1935, Death after injection of atebrin musonate. *Lancet, ii.*, 1056.

FISCHER, O., 1936, Beobachtungen bei der Malaria-prophylaxie mit Atebrin. *Arch. Schiffs- u. Tropenhyg.*, XL., 397.

FISCHL, V., and SINGER, E., 1935, Die Wirkungsweise chemotherapeutisch verwendeter Farbstoffe. *Z. Hyg. InfektKr.*, CXVI., 348.

FOY, H., KONDI, A., and PERISTERIS, M., 1936, Studies on atebrin—a controlled field experiment to test the relapse value of atebrin. *Trans. R. Soc. trop. Med. Hyg.*, XXX., 109.

FULTON, J. D., 1937, Studies in the chemotherapy of malaria. The distribution of anti-malarial drugs between red cells and serum. *Ann. trop. Med. Parasit.*, XXXI., 7.

GANGULI, P., 1933, Treatment of malaria with atebrin with records of blood pressure and electrocardiogram. *Arch. Schiffs- u. Tropenhyg.*, XXXVII., 413.

GENTZKOW, C. J., 1938, A method for the determination of atabrine in the blood. *Amer. J. trop. Med.*, XVIII., 149.

GREEN, R., 1932, A report on fifty cases of malaria treated with atebrin. A new synthetic drug. *Lancet, i.*, 826.

HAY, D. C., SPAAR, A. E., and LUDOVICI, H. L., 1935, Atebrin treatment in malaria. *Indian med. Gaz.*, LXX., 678.

HECHT, G., 1933, Pharmakologisches über Atebrin. *Arch. exp. Path. Pharmak.*, CLXX., 328.

HECHT, G., 1935, Experimentelle Untersuchung von Zirkulationsstörungen durch Plasmochin und Atebrin. Erwiderung auf die Arbeit von de Langen und Storm. *Klin. Wschr.*, XIV., 714.

HECHT, G., 1936, Die Verteilung des Atebrins im Organismus. *Arch. exp. Path. Pharmak.*, CLXXXIII., 87.

HICKS, E. P., 1935, Atebrin musonate : a note on the rate of absorption and on the local effects of intramuscular injection. *Rec. Malar. Surv. India*, V., 203.

JAMES, S. P., 1932, Synthetic antimalarial remedies and quinine. *Trans. R. Soc. trop. Med. Hyg.*, XXVI., 105.

JAMES, S. P., 1934, The direct effect of atebrin on the parasites of benign tertian and quartan malaria. *Ibid.*, XXVIII., 3.

JAMES, S. P., 1936, Consecutive reports on the anti-malarial chemotherapeutic tests carried out at the Devon Mental Hospital from 1932 to 1935. Devon Mental Hospital Printing Press, Exminster.

JARVIS, O. D., 1932, Results of treatment of malaria by (*a*) plasmoquine and quinine and (*b*) atebrin, together with some observations on malaria convalescents. *J. R. Army med. Corps.*, LIX., 190, 252.

KEHAR, N. D., 1935[1], Observations on the absorption and excretion of atebrin. *Rec. Malar. Surv. India*, V., 393.

KEHAR, N. D., 1935[2], The influence of food in the stomach on the absorption and excretion of atebrin. *Ibid.*, V., 405.

KIKUTH, W., 1932, Zur Weiterentwicklung synthetisch dargestellter Malariamittel. I. Ueber die chemotherapeutische Wirkung des Atebrin. *Dtsch. med. Wschr.*, LVIII., 530.

KINGSBURY, A. N., 1934. Psychoses in cases of malaria following exhibition of atebrin. *Lancet, ii.*, 979.

MAGIDSON, O. J., and GRIGOROWSKY, A. M., 1936, Acridin-Verbindungen und ihre Antimalaria-Wirkung. (I. Mitteil.) *Ber. dtsch. chem. Ges.*, LXIX., 396.

MASSA, M., 1933, Presenza ed eliminazione prolungata dell'atebrin demostrabile alla luce de Wood. *Rif. med.*, XLIX., 980.

MAUSS, H., and MIETSZCH, F., 1933, Atebrin, ein neues Heilmittel gegen Malaria. *Klin. Wschr.*, XII., 1276.

MIETZSCH, F., MAUSS, H., and HECHT, G., 1936, Experimental studies on atebrin. *Indian med. Gaz.*, LXXI., 521.

MOSNA, E., 1936, L'azione della chinina e dell'atebrin sul'*Plasmodium knowlesi*. *Riv. Malariol.*, XV., 99.

MÜHLENS, P., 1932, Die synthetischen Malariamittel Plasmochin und Atebrin. *Münch. med. Wschr.*, LXXIX., 537.

PETER, F. M., 1932, Zur Weiterentwicklung synthetisch dargestellter Malariamittel. III. Ueber die Wirkung des Atebrin gegen natürliche Malariainfektion. *Dtsch. med. Wschr.*, LVIII., 533.

RUBINSTEIN, B. N., 1936, Die Behandlung der Impfmalaria bei Paralytikern mit dem neuen synthetischen Präparat Acrichin. *Arch. Schiffs- u. Tropenhyg.*, XL., 167.

SCHECHTER, A. J., and TAYLOR, H. M., 1936, Atabrine pigmentation. *Amer. J. med. Sci.*, CXCII., 645.

SIOLI, 1932, Zur Weiterentwicklung synthetisch dargestellter Malariamittel. II. Ueber die Wirkung des Atebrin bei der Impfmalaria der Paralytiker. *Dtsch. med. Wschr.*, LVIII., 531.

SOESILO, R., GILBERT, A. P. W., and BANGINDO, Z. G. S., 1933, Atebrin in prophylaxis and therapy of malaria. *Geneesk. Tijdschr. Ned.-Ind.*, LXXIII., 153.

STORM, C. J., 1935, Ueber die Anwendung des Suprarenins bei intravenöser Injektion von Atebrin im Affenversuch. *Klin. Wschr.*, XIV., 756.

THONNARD-NEUMANN, E., and LE DOUX, H. A., 1931, The treatment of malaria with erion (atebrin), a new synthetic drug. Report of 75 cases. United Fruit Co. 20th Ann. Rep., Boston, Mass., p. 57.

TROPP, C., and WEISE, W., 1933, Untersuchungen über die Ausscheidung von Atebrin durch Harn und Fäzes. *Arch. exp. Path.*, CLXX., 339.

UDALAGAMA, L., 1935, Mental derangement in malaria cases treated by atebrin musonate injections. *Indian med. Gaz.*, LXX., 679.

VAN HEUKELOM, A. S., and OVERBEEK, J. G., 1936, Behandeling van de acute malaria-aanval met atebrine pro injectione. *Geneesk. Tijdschr. Ned.-Ind.*, LXXVI., 2507.

VAN SLYPE, W., 1936, Atébrine injectable et paludisme aigu. *Ann. Soc. belge Méd. trop.*, XVI., 429.

VARDY, E. C., 1935, Notes on clinical investigation of treatment of malaria by atebrin musonat injections. *Malay. med. J.*, X., 67.

WATS, R. C., and GHOSH, B. N., 1934, Quantitative and qualitative methods for detection of atebrin in urine. *Rec. Malar. Surv. India*, IV., 367.

WEISE, W., 1937, Untersuchungen ueber die Resorption und Ausscheidung des Atebrins. I. Methoden zur Bestimmung des Atebrins in Harn, Stuhl und Blut. *Arch. Schiffs- u. Tropenhyg.*, XLI., 715.

ACRIDINE DERIVATIVES

The therapeutic activity on malaria of atebrin has naturally led to an investigation of other acridine-containing compounds. In the U.S.S.R., Rubinstein (1936) has studied the effect of "acrichin" or "acriquine" on 21 cases of induced malaria. Patients were given 0·1 gm. by mouth three times a day for seven days and in all cases the attack was brought to an end. For intravenous or intramuscular injection 0·75 gm. of acriquine lactate was dissolved in 25 c.c. of a 3·4 per cent. solution of glucose. The quantity injected was from 5 to 8 c.c. "Tebetren" is said to be a mixture of hydroquinine, acriflavine, bile salts and acids. Any value it possesses appears to be largely due to its hydroquinine content, and, as Green (1934) has shown, its effect on the relapse rate is no better than that of quinine, while its cost is considerably greater (*cf.* Manson, 1936).

References

GREEN, R., 1934, Lectures on the development and use of the synthetic anti-malarial drugs. *Bull. Inst. med. Res. F.M.S.*, No. 2.

MANSON, D., 1936, Tebetren treatment in malaria. *Rec. Malar. Surv. India*, VI., 19.

RUBINSTEIN, B. N., 1936, Die Behandlung der Impfmalaria bei Paralytikern mit dem neuen synthetischen Präparat Acrichin. *Arch. Schiffs- u. Tropenhyg.*, XL., 167.

QUININE-ATEBRIN, QUININE-PLASMOQUINE AND ATEBRIN-PLASMOQUINE COMBINATIONS IN THE TREATMENT OF HUMAN MALARIA

(i.) At present there is little or no indication that the combination of quinine and atebrin is of value in treatment, while further observations are required to determine the value, if any, of the two drugs when administered one after the other.

(ii) The association of quinine with plasmoquine represents one of the most efficacious methods of treating benign tertian and quartan malaria. Treatment with average doses (1 to 1·30 gm.) of quinine plus plasmoquine in doses of only 0·02 to 0·03 gm. twice a week, greatly reduce, probably more than any other method, the number of relapses in infections with the majority of strains of benign tertian parasites, and in some cases also in malignant tertian infections. While the two drugs are often given simultaneously, some workers believe that it is far preferable to administer the drugs consecutively. The Malaria Commission of the League of Nations believe, however, that for the treatment of adult groups under observation there is no serious disadvantage to be feared from the simultaneous use of quinine and plasmoquine, more especially as this shortens the duration of treatment.

(iii) The combination of atebrin and plasmoquine is much more toxic than that of quinine and plasmoquine, and in fact the simultaneous use of atebrin and plasmoquine appears to aggravate the toxicity of each and is therefore inadvisable. Consecutive treatment with atebrin (0·30 gm. daily for five or seven days) followed by 0·02 gm. plasmoquine daily for five days has no appreciable effect either on the clinical manifestations or on the number of trophozoites in the blood. It does, however, reduce the number of relapses both in malignant tertian and, more especially, in benign tertian and quartan infections and, like the quinine *plus* plasmoquine treatment, it decreases and devitalises the gametocytes, especially those of *P. falciparum.* Clark and Komp (1937) believe that the action of atebrin and plasmoquine is superior to that of quinine and plasmoquine in reducing the parasite rate during an interepidemic period.

Reference

CLARK, H. C., and KOMP, W. H. W., 1937, A sixth year's report on malaria in Panama (Chagres Valley) with reference to drug control. *Amer. J. trop. Med.*, XVII., 59.

THE EFFECT OF ANTI-MALARIAL DRUGS ON THE INFECTIVITY OF THE PARASITES

If mass prophylaxis is to be successfully carried out by chemotherapeutic drugs it is essential that parasites in the blood should be rapidly rendered non-infective to anopheline mosquitoes. Comparatively few experiments have been carried out on this question, and it is obvious that results may differ with different mosquitoes and different species and strains of parasites. The most successful results have been obtained with plasmoquine. Barber and Komp (1927) showed that after two days' consecutive treatment with plasmoquine and quinine the blood of patients harbouring gametocytes of malignant and benign tertian malarial parasites is no longer infective for mosquitoes. Barber, Komp and Newman (1929) and Whitmore (1929) found that while a single dose of 0·2 mgm. of plasmoquine was insufficient, one of 0·3 mgm. rendered all crescents in the blood non-infectious for *Anopheles albimanus*. Amies (1930) showed that 0·04 gm. of plasmoquine in two equal doses of 0·02 gm. sixteen hours apart rendered crescent carriers non-infective for at least three days. Chopra and Basu (1937) showed that even a single dose of 0·02 gm. of plasmoquine was sufficient to prevent the development of crescents in *A. stephensi*. Plasmoquine in the same dose could not, however, inhibit the gametocytes of *P. vivax* in the same mosquito. Atebrin was shown to be without effect on the infectivity of crescents by Strickland and Roy (1932) and Chopra and Basu (1937), but three doses of atebrin inhibited development of the gametocytes of *P. malariæ* in *A. stephensi*. Quinine sulphate was found by Green (1929 and 1934) to be without effect on the infectivity of crescent-containing blood, while the gametocytes of *P. vivax* and *P. malariæ* were also unaffected. Chopra and Basu (1937), however, obtained evidence that the development of *P. vivax* was prevented in *A. stephensi*.

References

AMIES, C. R., 1930, The use of plasmoquine in subtertian malaria. *Bull. Inst. med. Res. F.M.S.*, No. 5.

BARBER, M. A., and KOMP, W. H. W., 1927, The viability of gametocytes in the blood of plasmochin-treated patients. *16th Ann. Rep. med. Dept. Unit. Fruit Co.*, p. 60.

BARBER, M. A., KOMP, W. H. W., and NEWMAN, B. M., 1929, The effect of small doses of plasmochin on the viability of gametocytes of malaria as measured by mosquito infection experiments. *U.S. pub. Hlth. Rep.*, XLIV., 1409.

CHOPRA, R. N., and BASU, B. C., 1937, A preliminary report on the effect of anti-malarial drugs upon the infectivity of the patients to the mosquitoes. *Indian J. med. Res.*, XXV., 459.

GREEN, R., 1929, Observations on some factors influencing the infectivity of malarial gamete carriers in Malaya to *Anopheles maculatus*. *Bull. Inst. med. Res. F.M.S.*, No. 5.

GREEN, R., 1934, Lectures on the development and use of the synthetic anti-malarial drugs. *Ibid.*, No. 2.

STRICKLAND, C., and ROY, D. N., 1932, Atebrin in malaria. *Indian med. Gaz.*, LXVII., 597.

WHITMORE, E. R. (1929), The place of plasmoquine in malaria control. 18*th Ann. Rep. med. Dept. Unit. Fruit. Co.*, p. 30.

SULPHONAMIDE COMPOUNDS

In view of the large number of different species of bacterial infections which have been successfully treated with prontosil and other sulphonamide compounds, it is not surprising that these drugs should have been tested in malarial infections. The first observations were made by Díaz de León (1937), who claimed to have successfully treated 15 cases with rubiazol. These results were confirmed by Hill and Goodwin (1937), who treated seven patients infected with *P. vivax* and ninety-three with *P. falciparum*. Intramuscular injections of 10 c.c. of prontosil were given every twelve hours. After four injections marked improvement occurred. No relapses are recorded, but there are said to have been two reinfections. The more severe the symptoms the more quickly did they yield to prontosil. No reactions were observed. Read and Pinto (1938) were unable to confirm these results. Further observations show that sulphanilamide, though it may reduce the temperature, is without effect on the parasites of either human or bird malaria. Chopra and Das Gupta (1938) believe, however, that soluseptasine has a specific action on *P. knowlesi* infections in monkeys; Rodhain (1938) and Coggeshall (1938) obtained similar results with sulphanilamide.

References

CHOPRA, R. N., and DAS GUPTA, B. M., 1938, A note on the therapeutic efficiency of soluseptasine in simian malaria (*P. knowlesi*). *Indian med. Gaz.*, **LXXIII.**, 395.

COGGESHALL, L. T., 1938, Prophylactic and therapeutic effect of sulphonamide compounds in experimental malaria. *Proc. Soc. exp. Biol. N.Y.*, **XXXVIII.**, 732.

DÍAZ DE LEÓN, A., 1937, Treatment of malaria with sulfonamide compounds. *Publ. Hlth. Bull.*, **LII.**, 1460.

HILL, R. A., and GOODWIN, JR., M. H., 1937, Prontosil in the treatment of malaria. *Sth. med. J.*, **XXX.**, 1176.

READ, H., and PINTO, J. O., 1938, Versuche mit den Sulfonamid-Präparaten bei der Malariabehandlung. *Arch. Schiffs- u. Tropenhyg.*, **XLII.**, 132.

RODHAIN, J., 1938, Paraaminophenylsulfamide et plasmodium des singes. *Ann. Soc. belge Med. trop.*, **XVIII.**, 255.

BLACKWATER FEVER

The ætiology of blackwater fever is still unknown, although there is general agreement that certain strains of malignant tertian malaria are almost always one of the predisposing causes. A number of factors appear to precipitate the acute attack which Fernán-Núñez (1936) regards as almost certainly anaphylactic in character; among these predisposing causes are exposure, intercurrent disease or the action of quinine which, by its effect on the parasites, is thought to liberate the allergen in intoxicating amounts. Fairley and Bromfield (1934) believe that the hæmolytic agent in blackwater fever arises during chronic subtertian malarial infection from some metabolic breakdown which is precipitated by the administration of quinine or plasmoquine. Nocht and Kikuth (1929), for instance, found that quinine salts were hæmolytic *in vivo* when given in association with small doses of hæmolytic substances, which in themselves are insufficient to produce either hæmolysis or hæmoglobinuria. Despite the alleged precipitating action of quinine, a very large number of cases of blackwater fever with parasites in the blood have been successfully treated by quinine. Considerable interest has been aroused by the question whether plasmoquine and atebrin are capable of provoking hæmoglobinuria. Owing to its methæmoglobin-producing action plasmoquine should possibly be regarded as one of the specific drugs capable of bringing on attacks of hæmoglobinuria. With regard to the action of atebrin there is greater uncertainty. Blackwater fever has, however, been recorded as occurring after the administration of atebrin in West Africa by Moir (1934), and in Greece by Foy and Kondi (1937), while Banerjee and Brahmachari (1933) and Chopra and Chaudhuri (1935) described cases in India in which symptoms of blackwater fever appeared after the administration of atebrin and plasmoquine.

For the present, therefore, it is not possible to assert with any certainty that atebrin and plasmoquine are without danger in precipitating an attack of blackwater fever.

Up to the present little progress has been made in the chemotherapeutic treatment of blackwater fever. Recently, however, Wats and Harbhagwan (1937) have found that methylene blue prevents the development of hæmoglobinuria in the rhesus monkey infected by *Plasmodium knowlesi*. When the infection is at its height quinine sulphate administered alone may expedite the onset of hæmoglobinuria which may or may not be present in untreated cases. The administration of methylene blue alone prevents the development of hæmoglobinuria but has no effect on the plasmodia. Intravenous injections of 5 c.c. of a 1 per cent. solution of methylene blue, together with quinine sulphate, on the other hand, cures the infection, the combination of drugs being more potent than quinine itself. The action of methylene blue as a preventive of blackwater fever in man appears worthy of investigation.

References

BANERJEE, N. G., and BRAHMACHARI, P., 1933, The occurrence of hemoglobinuria during treatment of malarial fever with atebrin and plasmoquine. *Indian med. Gaz.*, **LXVIII.**, 149.

CHOPRA, R. N., and CHAUDHURI, R. N., 1935, Some observations on the toxicity of synthetic anti-malarial remedies. *Ibid.*, **LXX.**, 1.

FAIRLEY, N. H., and BROMFIELD, R. J., 1934, Laboratory studies in malaria and blackwater fever. Part III. A new blood pigment in blackwater fever and other biochemical observations. *Trans. R. Soc. trop. Med. Hyg.*, **XXVIII.**, 307.

FERNÁN-NÚÑEZ, M., 1936, Hemoglobinuric fever: is it an allergic phenomenon? *Amer. J. trop. Med.*, **XVI.**, 563.

FOY, H., and KONDI, A., 1937, Three cases of blackwater fever following the oral administration of atebrin. *Trans. R. Soc. trop. Med. Hyg.*, **XXXI.**, 99.

MOIR, K. T., 1934, Blackwater fever following atebrin. *W. Afr. med. J.*, **VII.**, 121.

NOCHT, B., and KIKUTH, W., 1929, Über hämolytische Chininwirkungen. *Arch. Schiffs- u. Tropenhyg.*, **XXXIII.**, 355.

WATS, R. C., and HARBHAGWAN, S. A. S., 1937, A note on the prevention of hæmoglobinuria in *P. knowlesi* infections in *S. rhesus* by methylene blue and its curative value when combined with quinine salts. *Rec. Malar. Surv. India*, **VII.**, 179.

CHAPTER V

THE CHEMOTHERAPY OF TRYPANOSOMIASIS

Trypanocidal action, so far as it can be judged from the effects on the trypanosome infections of small animals, is a property possessed by a wide variety of chemical compounds, both metallic and non-metallic, having no obvious chemical or physical properties in common. Nevertheless the number of compounds actually in use in the treatment of trypanosomiasis of man and animals is comparatively small: tryparsamide and orsanine (*cf.* p. 159) among the quinquevalent arsenicals, germanin and, for animals, surfen C (*cf.* p. 177) practically exhaust the list. Even these few compounds possess serious drawbacks. The quinquevalent arsenicals are of doubtful value in early cases, and in late cases are liable to give rise to optic atrophy, while germanin is valueless in cases where the central nervous system is involved. There is thus need for further improvement in the chemotherapeutic treatment of trypanosomiasis, while in the trypanosomiasis of the New World no drug has yet been found with any certain curative action.

METAL-CONTAINING COMPOUNDS

A. Arsenical Preparations

TRYPARSAMIDE

Tryparsamide (tryparsone), which now finds a place in the Addendum (1936) to the British Pharmacopœia of 1932, is sodium N-phenylglycineamide-*p*-arsonate and should contain not less than 25·1 per cent. and not more than 25·5 per cent. of arsenic in organic combination, together with not less than 9·25 per cent. and not more than 9·5 per cent. of nitrogen, both figures being calculated with reference to the substance dried at 110° C. No biological test of toxicity is officially prescribed in Great Britain, but the following method is usually employed :—

Five well-nourished adult male rabbits are injected intravenously with a dose of 0·75 gm. of tryparsamide per kilogram of body weight in a 10 per cent. aqueous solution. Three of the five animals should survive without signs of serious intoxication. The period for which the animals are observed is seven days, but Launoy and Prieur (1935) prefer twenty days. Pottier and van den Branden (1935) suggest 1·0 gm. in place of 0·75 gm. per kilogram of body weight as the test dose but this modification is not generally employed. A further test for toxicity is recommended by van den Branden and Pottier (1938) in which ten white mice of 20 gm. weight are injected subcutaneously with 0·09 gm. of tryparsamide dissolved in ten times its weight of distilled water at a temperature of 20° C. Not more than four of the ten mice should develop nervous symptoms and at least six should survive for ten days.

Apart from occasional cases of erythema and very rarely of exfoliative dermatitis, the only toxic reaction commonly encountered is optic atrophy. Jamot (1929) observed 323 patients with ocular trouble among 25,638 treated with tryparsamide in the Cameroons. Van den Branden and Appelmans (1935) observed 24 cases among 118 patients. Van Hoof (1933) believes that the combination of germanin with tryparsamide increases the tendency to optic atrophy. Sloan and Woods (1937) distinguish two types of objective reaction due to tryparsamide. The acute type, which is rare, is characterised by rapid loss of vision, followed by almost complete blindness. After forced spinal drainage, however, the visual fields and vision may return almost to normal. The chronic reaction is characterised by contraction of the visual fields with retention of normal central vision and absence of objective signs of atrophy of the optic nerves. There is a marked tendency towards gradual recovery if tryparsamide is discontinued. Sensitivity to light of the central retina is reduced in patients showing a severe reaction, but in reactions of moderate severity the central sense of light is usually unaffected. For patients with normal optic nerves and fields of vision before treatment, tryparsamide is unlikely to cause serious ocular damage, provided the drug is permanently discontinued at the first appearance of defect in the visual fields.

Histologically Leinfelder (1938) finds acute degeneration of the retinal ganglion cells and of the innermost portion of the inner nuclear layer of the retina.

Guyomar'ch (1932) has not been deterred from using tryparsamide, apparently with success, in patients already affected with bilateral optic atrophy and meningitis due to the trypanosome infection. Raingeard (1934) believes that fifteen intravenous injections of 10 c.c. of a 20 per cent. aqueous solution of sodium hyposulphite given on alternate days greatly benefit patients with ocular involvement. Evidence has been brought forward by Barlovatz (1933[1]) to suggest that in some cases tryparsamide stored in the tropics for as long as three years may show a greater tendency to produce blindness. This observer, however, found that his samples of tryparsamide also caused sudden death an hour or two after injection.

The value of tryparsamide in the treatment of trypanosomiasis lies in the ease with which it penetrates the central nervous system in a trypanocidal form. It has been claimed that hexamethylenetetramine increases this trypanocidal power, but van den Branden and Pottier (1934) failed to obtain any evidence of such action. Tryparsamide has been used in the treatment of first stage cases, and according to Chesterman (1932) six to eight weekly injections should be given. For children the dose should be 0·07 gm. per kilogram of body weight, for young adults 0·055 gm. per kilogram of body weight, and for adults 0·045 gm. per kilogram of body weight. Second courses are not recommended. In second stage cases the same number of injections is given but the doses are larger, 0·09 gm for children, 0·07 gm. for young adults and 0·06 gm. per kilogram for adults. The maximum dose for adults should be 4 gm. In advanced second stage cases two or three large doses should be given at five days' interval followed by a rest of ten to fourteen days. Relapses are associated with some degree of resistance to tryparsamide and, according to Maclean and Fairbairn (1932), relapse cases are particularly susceptible to the toxic action of tryparsamide. Loré and Marty (1933) find that patients who have passed into the second stage of the disease, after having received a course of tryparsamide, furnish the greatest proportion of deaths. On the contrary, patients in the nervous stage who have not previously received tryparsamide stand a reasonable chance of cure even though they have been treated with other arsenicals such as atoxyl and tryparsamide. This curious fact, for which no obvious explanation is yet forthcoming, has also been observed by Chesterman (1932). The percentage of cures produced by tryparsamide has varied greatly. Chesterman (1932) believes that 50 per cent. of second stage patients may be saved, while Vamos (1936) among 1,676 patients had 15·6 per cent. of deaths and 9·01 per cent. of failures.

A study of the cell content of the cerebrospinal fluid is of importance in determining the prognosis. As a rule tryparsamide rapidly reduces the number of cells in the cerebrospinal fluid (Laurent, 1932), but a raised cell content a few weeks after treatment is not an indication of failure, since the cell content may still return to normal as a result of the treatment. On the other hand tryparsamide may on occasions induce a lymphocytosis in a normal cerebrospinal fluid (Barlovatz, 1933[2]), or, more rarely, give rise to a Jarisch-Herxheimer reaction in the central nervous system (Mackie, 1935).

References

BARLOVATZ, A., 1933[1], Sur certains accidents de cures au tryponarsyl. *Bull. Soc. Path. exot.*, XXVI., 850.

BARLOVATZ, A., 1933[2], Des réactions meningées consécutives au traitement dans la maladie du sommeil. *Brux. méd.*, XIII., 659.

CHESTERMAN, C. C., 1932, Some results of tryparsamide and combined treatment of Gambian sleeping sickness. *Trans. R. Soc. trop. Med. Hyg.*, XXV., 415.

GUYOMAR'CH, 1932, Méningite trypanosomiasique avec amaurose guérie par la tryparsamide. *Marseille. méd.*, LXIX., 822.

JAMOT, E., 1929, La maladie du sommeil au Cameroun en janvier 1929. *Bull. Soc. Path. exot.*, XXII., 473.

LAUNOY, L., and PRIEUR, M., 1935, Contribution à l'étude de l'essai biologique de la tryparsamide. *Ibid.*, XXVIII., 389.

LAURENT, R., 1932, Remarques à propos de l'action du traitement par la tryparsamide, sur la lymphocytose du liquide céphalorachidien, au cours de la trypanosomiase. *Ann. Soc. belge. Méd. trop.*, XII., 539.

LEINFELDER, P. J., 1938, Pathologic changes in amblyopia following tryparsamide therapy. *J. Amer. med. Ass.*, CXI., 1276.

LORÉ, A., and MARTY, J., 1933, De l'efficacité de la tryparsamide chez les trypanosomés en deuxième période. *Bull. Soc. Path. exot.*, XXVI., 959.

MACKIE, F. P., 1935, The Jarisch-Herxheimer reaction in trypanosomiasis, with a note on the morular cells of Mott. *Trans. R. Soc. trop. Med. Hyg.*, **XXVIII.**, 377.

MACLEAN, G., and FAIRBURN, H., 1932, Treatment of Rhodesian sleeping sickness with Bayer 205 and tryparsamide : observations on 719 cases. *Ann. trop. Med. Parasit.*, **XXVI.**, 157.

POTTIER, R. and VAN DEN BRANDEN, F., 1935, Contribution à l'étude toxicologique de glyphénarsine belge IV (tryparsamide, tryponarsyl, novatoxyl, trypotan). *Bull. Soc. Path. exot.*, **XXVIII.**, 726.

RAINGEARD, 1934, Traitement par l'hyposulfite de soude des troubles oculaires dus aux trypanocides. *Rev. Mèd. et Hyg. trop.*, **XXVI.**, 143.

SLOAN, L. L., and WOODS, A. C., 1937, The effect of tryparsamide on the eye : a clinical study of the objective ocular reaction. *Amer. J. Syph.*, **XX.**, 583.

VAMOS, S., 1936, Traitement de trypanosomés dans un secteur du Moyen-Chari (A.E.F.). Étude de 3,705 observations. *Bull. Soc. Path. exot.*, **XXIX.**, 1015.

VAN DEN BRANDEN, F., and APPELMANS, M., 1935, Les troubles visuels au cours du traitement de la trypanosomiase humaine par la tryparsamide (tryponarsyl, trypotan, novatoxyl). *Brux. méd.*, **XV.**, 1405.

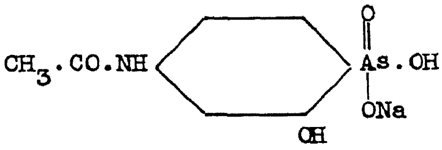
VAN DEN BRANDEN, F., and POTTIER, R., 1934, L'hexaméthylène tétramine associée à la tryparsamine dans le traitement de la trypanosomiase.—Contrôle biologique du tryponurile. *Ann. Soc. belge Méd. trop.*, **XIV.**, 499.

VAN DEN BRANDEN, F., and POTTIER, R., 1938, Le contrôle biologique des glyphenarsines (tryparsamide, tryponarsyl, novatoxyl, trypothan). *Bull. Soc. Path. exot.*, **XXXI.**, 46.

VAN HOOF, L., 1933, Un nouveau dérivé antimonié organique, le Dn 18, dans la trypanosomiase humaine. *Ann. Soc. belge Méd. trop.*, **XIII.**, 479.

Sodium-4-acetylamino-2-hydroxyphenylarsonate

Fourneau 270, or orsanine, was originally studied by Fourneau, Navarro-Martin, Tréfouel and Tréfouel (1923), who found it to have a curative action in experimental nagana infections in mice, its chemotherapeutic index being 1 : 20. It has been used clinically by Ledentu and Daude (1926), Ledentu and Vaucel (1927), and more recently Sicé (1930 and 1931) and other French workers.



Sodium-4-acetylamino-2-hydroxyphenylarsonate.

(Orsanine)

The general conclusion reached by them is that it is equal, or superior, to tryparsamide in the treatment of patients at all stages of the disease, and it is now extensively used in French Equatorial Africa, where it has partially replaced tryparsamide. Its dosage, toxicity and action on the optic nerve are closely similar to those of tryparsamide. According to Hawking, Hennelly and Quastel (1937), the power of orsanine to render the cerebrospinal fluid trypanocidal, after intravenous injection, is as great as, if not greater than, that of tryparsamide. The greater activity of orsanine is not due to its greater penetration into the cerebrospinal fluid, but to the larger proportion which is reduced within a given time to the active form.

References

Fourneau, E., Navarro-Martin, A., Tréfouel, M. and Mme., 1923, Les dérivés de l'acide phénylarsinique (arsenic pentavalent) dans le traitement des trypanosomiases et des spirilloses expérimentales. Relation entre l'àction thérapeutique des acides arsiniques aromatiques et leur constitution. *Ann. Inst. Pasteur*, **XXXVII.**, 551.

Hawking, F., Hennelly, T. J., and Quastel, J. H., 1937, Trypanocidal activity and arsenic content of the cerebrospinal fluid after administration of arsenic compounds. *J. Pharmacol.*, **LIX.**, 156.

Ledentu, G., and Daude, J., 1926, Essai du traitement de la trypanosomiase humaine par le 270 Fourneau. *Ann. Inst. Pasteur*, **XL.**, 830.

Ledentu, G., and Vaucel, M., 1927, Note complementaire sur le "270 Fourneau" en trypanosomiase humaine. *Ibid.*, **XLI.**, 1233.

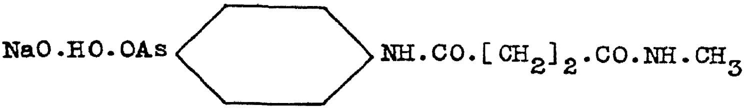
Sicé, A., 1930, Contribution à l'étude du 270 Fourneau appliqué au traitement de la trypanosomiase humaine. *Ibid.*, **XLIV.**, 221.

Sicé, A., 1931, L'Institut Pasteur de Brazzaville et la trypanosomiase humaine. *Bull Soc. Path. exot.*, **XXIV.**, 5.

Neocryl

In order to attempt to combine the desirable features of acetyl-*p*-arsanilic acid and tryparsamide an homologous series of compounds of the general formula

were prepared by Morgan and Walton (1931). Among these compounds sodium succinanilomethylamide-*p*-arsonate ($n = 2$; $R_1 = H$; $R_2 = CH_3$), "neocryl," was selected for further study by Yorke and his colleagues (1936).



Sodium succinanilomethylamide-p-*arsonate*

The maximum tolerated dose of neocryl for a 20 gm. mouse is 40 to 50 mgm., the minimal effective for mice infected with *T. rhodesiense* 6 mgm. per 20 gm. of body weight and the minimum curative dose 12·5 mgm., as compared with 20 mgm. of tryparsamide per 20 gm. of body weight. In rabbits a dose of 1 gm. per kilogram of body weight killed four out of seven rabbits. Trypanocidal action has been shown by Morgan and Walton (1937) to be enhanced by an amide group in the para-position in phenylarsonic acid.

In man neocryl is well tolerated in weekly doses of from 2 to 4 gm. The drug has been used in the treatment of syphilis as well as in trypanosomiasis. Yorke and his colleagues (1936) treated 12 cases of Nigerian sleeping sickness with promising results, while Acres (1937) has recorded his observations on 24 cases in the Belgian Congo. In first-stage cases there was definite clinical improvement and immediate effects, as determined by gland and lumbar puncture, were satisfactory, the results comparing favourably with those obtained with tryparsamide.

In second-stage cases, despite marked clinical improvement, the results were unsatisfactory and in three patients the drug failed, one remaining positive at the end of treatment and two others quickly relapsing. In only one instance was there impairment of vision, which persisted after the drug was stopped. Within six months two out of nine patients in the first stage and seven or eight out of twelve in the second stage had to receive further treatment.

In the Gambia, Murgatroyd (1937) treated 122 patients, of whom forty-four had normal and seventy-eight pathological cerebrospinal fluid. As a routine 10 doses of 45 mgm. per kilogram of body weight were given at weekly intervals, though in a few cases doses up to 60 mgm. per kilogram of body weight were given. The drug was injected intravenously in a 25 per cent. solution in freshly distilled water. Of the forty-four first-stage patients one patient relapsed, while two others exhibited visual impairment, though both recovered. Of forty-six second-stage patients completing a course of 10 or more injections, forty-five showed clinical improvement. Six patients suffered from toxic effects, three experienced visual disturbances, while others had transient dizziness, vomiting and abdominal pain. In addition to the four patients who responded imperfectly to treatment, four others are known to have relapsed at intervals of seven to fifteen weeks after completing a course of treatment. The results obtained are thus very similar to those seen after the use of tryparsamide and probably the two drugs are very similar in their actions. It is of interest to note, however, that Hawking, Hennelly and Quastel (1937) found that, though approximately the same amounts of arsenic are found in the cerebrospinal fluid after equal doses of neocryl or tryparsamide, the trypanocidal titre of the fluid is appreciable after tryparsamide but negligible after neocryl. It is suggested that the active compound found in the cerebrospinal fluid after a dose of tryparsamide is derived from a reduction of the drug occurring in the brain, whereas in the case of neocryl, although the drug reaches the brain, it is not converted into a trypanocidally-active compound.

References

ACRES, I. S., 1937, The treatment of sleeping sickness with neocryl. *Trans. R. Soc. trop. Med. Hyg.*, XXI., 333.

HAWKING, F., HENNELLY, T. J., and QUASTEL, J. H., 1937, Trypanocidal activity and arsenic content of the cerebro-spinal fluid after administration of arsenic compounds. *J. Pharmacol.*, LIX., 157.

MORGAN, G. T., and WALTON, E., 1931, New derivatives of *p*-arsanilic acid. Part I. : *p*-arsonosuccinanilic acid and its related compounds. *J. chem. Soc.*, LXXXIII., 615.

MORGAN, G. T., and WALTON, E., 1937, New derivatives of *p*-arsanilic acid. *Chem. Ind.*, LVI., 853.

MURGATROYD, F., 1937, Observations on the therapeutic action of three arsenicals, neocryl, K.324 and K.352, in Gambian sleeping sickness. *Ann. trop. Med. Parasit.*, XXXI., 473.

YORKE, W., MURGATROYD, F., GLYN-HUGHES, F., LESTER, H. M. O., and ROSS, A. O. F., 1936, A new arsenical for the treatment of syphilis and trypanosomiasis. *Brit. Med. J.*, *i.*, 1042.

DIARSONIC ACIDS

It has long been recognised that *Trypanosoma congolense* fails to react to the quinquevalent arsenicals which readily cure other trypanosome infections in small rodents. This property of *T. congolense* is not due to any drug fastness, for Fourneau *et al.* (1933) have been able to show that numerous compounds containing two arsonic acid groups can cure infections due to *T. congolense*. The chemotherapeutic indices of these compounds varied from 1 : 1 to 1 : 3; the most active compounds were derivatives of phenylenediarsonic acid of the type $NH_2C_6H_3 = (AsO_3H_2)_2$, derivatives of diphenyldiarsonic acid, derivatives of *p*-aminophenylarsonic acid such as the substituted carbamide $CO(NHC_6H_4AsO_3H_2)_2$ and azo-compounds of the type $R—N = N—C_6H_4—C_6H_4N = N—R$.

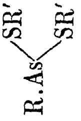
Reference

FOURNEAU, E., TREFOUËL, J., TREFOUËL, MME., BOVET, D., and KOETSCHET, P., 1933, Chimiothérapie des infections à *Trypanosoma congolense*. Action élective des composés organiques polyarsenicaux. *C. R. Acad. Sci.*, CXCVI., 1173.

ARYLTHIOARSINITES

In the thioarsinites tervalent arsenic is linked through sulphur to one, two or three organic radicals. A number of dithioarsinites capable of forming water-soluble sodium salts have been prepared by Cohen, King and Strangeways (1931 and 1932) by direct condensation of arylarsenious oxides with two molecular proportions of a thiol compound containing a carboxyl group. Starting with benzamide-*p*-arsenoxide and acetanilide-*p*-arsenoxide, two series of compounds, I and II, were prepared by employing in the condensations α-thiolacetic acid, β-thiolpropionic acid and its α-amino derivative, the naturally occurring amino-acid cysteine, *o*-thiolbenzoic acid, *m*-thiolbenzoic acid, α-thiolacetamide and glutathione.

THE TRYPANOCIDAL ACTION OF DITHIOARSINITES.

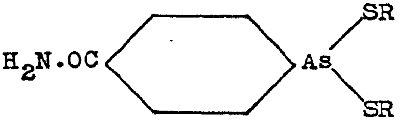


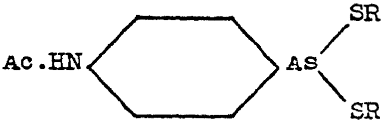
$R.As\begin{smallmatrix}SR'\\SR'\end{smallmatrix}$

(Cohen, King and Strangeways, 1931.)

(Molecular weight in brackets.)

Thiol constituent	Benzamide-*p*-arsenoxide (211)			Acetanilide-*p*-arsenoxide (225)		
	Maximum tolerated dose in mgm. per gram of body weight	Minimum curative dose	No. of days blood free from trypanosomes	Maximum tolerated dose in mgm. per gram of body weight	Minimum curative dose	No. of days blood free from trypanosomes
—	0·03	0·01	14	0·02	0·005	>30
Thiolacetic acid (76)	0·05	0·03	>30	0·05	0·01	>30
β-Thiolpropionic acid (88)	0·075	0·01	>30	0·05	0·005	>30
Cysteine (121)	0·1	0·01	>30	0·05	0·0075	>30
o-Thiolbenzoic acid (154)	0·075	0·01	>30	0·04	0·02	>30
m-Thiolbenzoic acid (154)	0·1	0·025	>30	0·1	0·01	>30
Glutathione (307)	0·35	0·1	19	0·2	0·075	11





II

On testing the trypanocidal activity of a number of these compounds against *T. equiperdum* infections in mice it was found that as the molecular weight of the thiol constituent was increased, the maximum tolerated dose, with one or two exceptions, also increased.

Of the arylthioarsinites prepared from cysteine and glutathione the most actively trypanocidal were found by Cohen, King and Strangeways (1932) to be di (β-amino-β-carboxyethyl) benzamide-*p*-thioarsinite $NH_2.CO.C_6H_4.As[S.CH_2.CH(NH_2)CO_2H]_2$, K 324, and diglutathionyl - 4 - acetamido - 2 - hydroxyphenylthioarsinite, K352.

References

Cohen, A., King, A., and Strangeways, W. I., 1931, Trypanocidal action and chemical constitution. Part X., Arylthioarsinites. *J. Chem. Soc.*, 3043.

Cohen, A., King, A., and Strangeways, W. I., 1932, Trypanocidal action and chemical constitution. Part XII. Arylthioarsinites from cysteine and glutathione. *Ibid.*, 2505.

K.324 and K.352

Of the aromatic thioarsinites prepared by Cohen, King and Strangeways (1932) two compounds, K.324, di-(β-amino-β-carboxyethyl)-benzamide-*p*-thioarsinite, and K.352, diglutathionyl-4-acetamido-2-hydroxyphenylthioarsinite, were found by Strangeways (1935) to be effective in curing *T. rhodesiense* and *T. gambiense* infections in mice in doses which were only a fraction of the maximum tolerated. Three to six doses of 0·01 gm. of K.324 per kilogram of body weight, or four to eight doses of 0·01 gm. of K.352 per kilogram of body weight, at three-day intervals, produced permanent cures in rabbits infected with a strain of *T. rhodesiense*. In man, however, Murgatroyd (1937) found that even with doses of only 2·5 mgm. per kilogram of body weight, vomiting and diarrhœa occasionally occurred, while with larger doses toxic albuminuria also was seen. In maximum tolerated doses no clinical improvement was noticed, but one patient after a single dose of 2·5 mgm. per kilogram of body weight of K.352 developed tachypnœa and hyperthermia and died.

References

Cohen, A., King, A., and Strangeways, W. I., 1932, Trypanocidal action and chemical constitution. Part XII. Arylthioarsinites from cysteine and glutathione. *J. Chem. Soc.*, 2505.

Murgatroyd, F., 1937, Observations on the therapeutic action of three arsenicals, neocryl, K.324 and K.352, in Gambian sleeping sickness. *Ann. trop. Med. Parasit.*, XXXI., 473.

Strangeways, W. I., 1935, Trypanocidal action of two arsenicals, K.324 and K.352, on infections in mice and rabbits. *Ann. trop. Med. Parasit.*, XXIX., 231.

OTHER ARSENICAL COMPOUNDS

Aromatic arsonic acids containing amide groups. Gough and King (1930) found that benzamide-*p*-arsonic acid was trypanocidally active in mice but produced very severe nervous symptoms. A series of arsonic or arsinous acids containing carboxyl or sulpho groups were, however, inactive but when converted into primary amides, carboxyamides or sulphonamides they became trypanocidally active in all cases. This is explained by the fact that the acids when containing sulpho or carboxyl-groups are rapidly excreted, whether reduced to the arsenoxides or not, since they contain solubilising groups. Those compounds containing amide groups will be excreted rapidly so long as they remain arsonic acids, but when once reduced to the arsinous acid stage excretion will be slow, since the solubilising group, the arsonic acid group, has disappeared.

" Arseno-Detoxin " Compounds. What are claimed to be compounds consisting of the condensation products of hydroxy-amino- and hydroxyaminobenzylarsenoxides and their substitution products with high molecular keratin hydrolysates have been studied by Collier and Verhoog (1937). One compound " As XIII " was found to have a chemotherapeutic index of 1 : 66·6 to 1 : 80 when given subcutaneously to mice infected with nagana. When given by mouth its chemotherapeutic index was only 1 : 10.

Arsenical derivatives of pyridine. Twenty-one arsenopyridine compounds and two arsenostibio derivatives were studied by Christison (1934) for their action on infections due to *T. brucei* and *T. lewisi*. One compound, BR 23, 2-pyridone-3-amino-5-arsonic acid had a weak action in *T. lewisi*. The arsenostibio-compound, Sdt 386B, so active in Bartonella infections, exerted a very slight action on *T. lewisi*. Lester (1935), in human cases infected with *T. gambiense*, found this compound less effective than germanin.

Arsenic-containing dyestuffs. Two arsenic-containing dyestuffs, arsenic yellow and arsenic brown, have been found by Fischl and Singer (1935) to be active in infections in mice due to *Spirochœta recurrentis* and *T. brucei*.

References

CHRISTISON, M. H., 1934, Ueber chemotherapeutische Versuche bei der Rattentrypanose (*Trypanosoma lewisi*). *Zbl. Bakt.*, Abt. I Orig., CXXXII., 228.

COHEN, A., KING, H., and STRANGEWAYS, W. I., 1931, Trypanocidal action and chemical constitution. Part XI. Aromatic arsenic acids containing amide groups. *J. Chem. Soc.*, 3236.

COLLIER, W. A., and VERHOOG, M. J., 1937, Ueber Arsen-Detoxin Verbindungen. *Z. ImmunForsch.*, XC., 43.

FISCHL, V., and SINGER, E., 1935, Chemotherapeutische Prüfung zweier arsenhaltiger Farbstoffe. *Biochem. Z.*, CCLXXVI., 277.

GOUGH, G. A. C., and KING, H., 1930, Trypanocidal action and chemical constitution. Part IX. Aromatic acids containing an amide group. *J. Chem. Soc.*, 669.

LESTER, H. M. O., 1935, Report of the Tsetse Investigation. Nigeria. Rep. Med. Hlth. Services for year 1934. Appendix B, p. 69.

B. Antimony Preparations

Antimony was first used in the treatment of trypanosomiasis by Plimmer and Thompson (1908) who found that the blood of laboratory animals infected with *T. brucei* or *T. evansi* was sterilised by injections of potassium or sodium antimonyl tartrate. Until recently antimony was almost the only compound used in the treatment of trypanosomiasis in domestic animals though numerous attempts have been made to introduce antimonial compounds which might prove suitable for the treatment of human trypanosomiasis.

Infections due to *T. vivax* and *T. uniforme* have been treated with success by antimosan (*cf.* p. 47), while the same drug has cured a sheep experimentally infected with *T. simiæ* (Hornby, 1938). Launoy and Prieur (1937) have studied the trypanocidal action of four new organic preparations of antimony, probably complex tartrates of the stibonic acids, and described as methylglucamine aminophenylstibonate, sodium diphenylcarbamidestibonotartrate, sodium *para*acetylaminophenylstibonotartrate, and sodium diphenylcarbamidodistibonopyrocatechol sulphonate. The only one of these with a curative action in infections due to *T. brucei* and *T. annamense* was methylglucamine aminophenylstibonate which contains 30·3 per cent. of antimony. To produce cures of infections due to *T. congolense* the amount of antimony required is double that necessary for the other trypanosomes. In addition to the methylglucamine salt, glucamine aminophenylstibonate was found to be active by Launoy (1937), who also investigated the trypanocidal action of lithium antimony thiomalate, a tervalent antimonial. This compound was found to be active in early infections of *T. annamense* in the mouse. In later infections cure was obtained in approximately 50 per cent. of mice, but in guinea-pigs the course of the disease was only prolonged and no cures were obtained. Among other tervalent antimonials antimosan has been used by Evans (1936) in the treatment of cattle infected by *T. congolense*. Injections, consisting of 10 c.c. per 100 lb. of body weight, were given once a week for five weeks and offered great hope of a complete cure being effected.

A number of antimonial compounds of hydroxyquinoline have been prepared in Belgium and a few of them have been tested in human trypanosomiasis. Four of the compounds Dn 9, Dn 12, Dn 17 and Dn 20 are described by Rodhain, Dubois and Hendrix (1933) as being quinquevalent antimonials, the remainder, Dn 7, Dn 8, Dn 11, Dn 14 and Dn 18, as tervalent. All the compounds proved trypanocidal, but apart from Dn 9 and Dn 18 all the preparations were so irritating that they could not be given subcutaneously to animals. Van Hoof (1933 [1]) treated twenty-six patients with Dn 12. Doses of 0·5 gm. were given intravenously twice weekly to a total amount of 5·0 to 10·0 gm. Many patients, however, were unable to tolerate such large doses and the individual dose had, therefore, to be reduced to 0·25 gm. Immediate shock, decreased, however, by a preliminary injection of 0·5 gm. of sodium thiosulphate, and poisonous symptoms as a result of accumulation of the drug were not uncommon, while dermatitis occurred in four of twenty-six patients. In some patients the trypanosomes were removed from the peripheral circulation and the cells in the cerebrospinal fluid were reduced in numbers. In others, however, no curative effect was produced.

Dn 18 is a tervalent antimonial and, since it is affected by steel, must be injected in a glass syringe with platinum or nickel needles. The drug was found by van Hoof (1933 [2]) to produce peripheral sterilisation in human cases a few minutes after a single dose. This sterilisation lasted for from seven to seventy-two days and no intolerance was observed in patients who were given a series of injections of 0·5 gm. twice weekly. As much as 10 gm. could be given intravenously without producing toxic dermatitis or other reactions. Dn 18 appears to have a definite curative value in advanced cases and is said to be of value in cases where the trypanosomes have become resistant to arsenic.

References

Evans, J. T. R., 1936, *Trypanosoma congolense* infection in cattle : treatment by antimosan and surfen " C." *J. comp. Path.*, **XLIX.**, 160.

Hornby, H. E., 1938, Pathology and therapeutics of nagana : an appeal. *Ibid.*, **L.**, 312.

Launoy, L., 1937, Du traitement de l'infection expérimentale à " Trypanosoma annamense " de la souris et du cobaye, par l'Sb.-III-thiomalate de lithium (Sb. trivalent) et par les sels de glucamine de l'acide amino-phénylstibinique (Sb. pentavalent). *Bull. Acad. Méd. Paris*, **CXVII.**, 743.

Launoy, L., and Prieur, M., 1937, Étude de l'action trypanocide comparée sur *Tr. brucei*, *Tr. congolense*, *Tr. annamense* normal et *Tr. annamense* chimiorésistant de quelques composés organiques d'antimoine. *Bull. Soc. Path. exot.*, **XXX.**, 869.

Plimmer, H. G., and Thompson, J. D., 1908, Further results of the experimental treatment of trypanosomiasis in rats. *Proc. R. Soc. London*, **B**, **LXXX.**, 1.

RODHAIN, J., DUBOIS, A., and HENDRIX, H., 1933, Essais thérapeutiques de nouveaux composés antimoniaux dérivés de la quinoléine (Dn 9 à Dn 20 U.C.B.). *Ann. Soc. belge Méd. trop.*, XIII., 17.

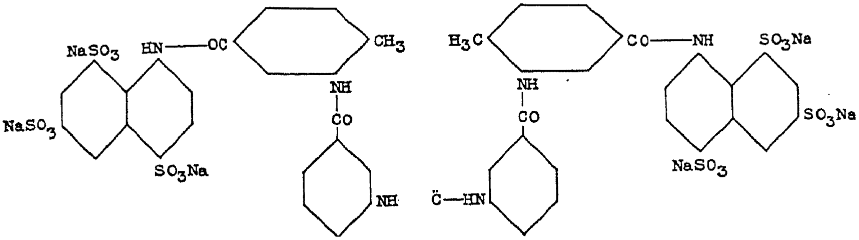
VAN HOOF, L., 1933^{1},E ssai d'un nouveau dérivé antimonié organique, Dn 12 dans la trypanosomiase humaine. *Ibid.*, XIII., 345.

VAN HOOF, L., 1933^{2}, Un nouveau dérivé antimonié organique le Dn 18, dans la trypanosomiase humaine. *Ibid.*, XIII., 479.

NON-METALLIC COMPOUNDS

GERMANIN (Bayer 205) or MORANYL (Fourneau 309)

The drug germanin (Bayer 205), now known in France as moranyl (Fourneau 309) and in Great Britain as antrypol, was introduced at the end of 1920. Its chemical constitution was given by Schlossberger (1928) as :—



Germanin

a structural formula identical with that arrived at by Fourneau (1924). It is of interest to note that as far as is at present known the slightest deviation from this formula is attended with a diminution in trypanocidal activity. This fact explains why other ureas of the aminonaphthalenesulphonic acid series have not found a place in the chemotherapy of trypanosomiasis.

Sufficient time has now elapsed since the introduction of germanin to indicate that it is a drug of the greatest value in the early stages of trypanosomiasis before the central nervous system is attacked. Thereafter it is of little value. This failure is almost certainly due to the fact that germanin does not readily penetrate into the central nervous system. Until recently, however, an accurate method for the estimation of germanin in the tissues was lacking. The method of estimation suggested by Steppuhn and Utkina-Ljubowzewa (1924), in which the products obtained by alkaline hydrolysis of germanin were coupled with *p*-diazobenzenesulphonic acid in acetic acid solution and compared with standards, was criticised by Lang (1931). He suggested the acid hydrolysis of germanin, the amines liberated being diazotised and coupled in acetic acid solution with α-naphthol. The orange yellow colour produced is estimated colorimetrically. Unfortunately, filtrates from urine, plasma and serum possess a yellow colour which cannot be completely removed by kaolin and tends to interfere with the accuracy of this method. Dangerfield, Gaunt and Wormall (1938), however, have found that methyl-α-naphthylamine yields a magenta colour when coupled in acetic acid solution with the diazotised products of hydrochloric acid hydrolysis of germanin. The results obtained by this method are discussed in relation to the chemoprophylaxis of trypanosomiasis.

Germanin has now been used in the treatment of many thousands of cases of sleeping sickness. Toxic reactions are remarkably few in number. Vomiting may occur if the drug is given too rapidly : transient œdema, pruritus, purulent conjunctivitis, stomatitis, skin rashes and mild peripheral neuritis have also been recorded. Lester (1935) records three deaths following sudden collapse, while anuria or the presence in the urine of albumen, casts, and in some cases blood, indicate that the drug must be given with caution to patients suffering from nephritis. Nevertheless, de Marqueissac (1933) found albumen in the urine of twenty-five of thirty-three untreated patients with trypanosomiasis. After the first few doses of germanin the albumen either completely disappeared or was greatly reduced in amount.

Provided that germanin is given in the first few weeks after the beginning of illness there is a reasonable possibility of cure in infections due to both *T. gambiense* and *T. rhodesiense*. Nevertheless approximately 25 per cent. of cases of infections due to *T. rhodesiense* of less than a month's duration relapse, while other cases of much longer duration are curable either by germanin alone or by combined treatment with germanin and tryparsamide. Fairbairn (1934) believes that in some patients, giving a short history of illness, the trypanosome infection causes early damage to the central nervous system : this is shown by a high percentage of protein in the cerebrospinal fluid. Such patients are not curable by germanin alone and possibly not curable at all. On the other hand, there are patients who give a history of being ill for more than one month and yet the percentage of protein in the cerebrospinal fluid is low, thus indicating an intact nervous system. Such patients are easily cured.

Germanin, or moranyl, has on occasions been used alone in the treatment of all stages of human sleeping sickness. Maclean (1929), for instance, gave 4 gm. of germanin in three or four doses spread over a month to patients recently infected with *T. rhodesiense.* To allow a margin of safety, however, as much as 8 gm. should be given and it is preferable to follow the germanin with tryparsamide in doses of 2 to 3 gm. at weekly intervals, until at least 36 gm. have been given. In a later communication, Maclean and Fairbairn (1932) reported the results of treatment of 719 patients who had been kept under observation for a minimum period of two years and five months. When 4 to 5 gm. of germanin were given in 1 gm. doses every week 73·5 per cent. of the patients remained well, provided treatment was begun within a month of the onset of symptoms. Although Maclean and Fairbairn were unable to obtain conclusive proof that the combined administration of germanin and tryparsamide gives better results in moderately advanced cases, nevertheless in late cases the combination is an advantage, since no cachetic cases ever recover with germanin alone, while a few undoubtedly improve with combined treatment. Coghlan (1933) gives 5 gm. of germanin, 1 gm. at weekly intervals, followed after a month's rest by at least 20 gm. of tryparsamide, 3 gm. at weekly intervals. This is preferred to 3 gm. of germanin, 1 gm. every alternate day, followed after a week's interval by a total of 10 gm. of tryparsamide, 3 to 4 gm. being given at weekly intervals. De Marqueissac (1933) believes that germanin and tryparsamide given immediately one after the other exert a definitely synergic action, while Lester (1935) also believes that germanin and tryparsamide are far preferable to either drug alone in the treatment of infections due to *T. gambiense*, although as a rule patients with *T. gambiense* infections are more readily cured than those with infections due to *T. rhodesiense.*

Even, however, when doses as large as 0·05 gm. of tryparsamide and 0·01 gm. of moranyl per kilogram of body weight are given each week for a period of twelve weeks, Millous (1936) in the Cameroons has found that a certain number of cases are not sterilised.

Although large doses of germanin, 18 gm. in six days, can be given by mouth, this method of administration rarely causes permanent cure, though temporary improvement may follow (Zschucke, 1936). In mice infected with *T. brucei*, inhalation of a 10 per cent. solution of moranyl in the form of an invisible cloud has been found by Trillat (1936) to prevent relapse during an observation period of a month.

References

Coghlan, B. A., 1933, Notes on one hundred and ten cases of Rhodesian sleeping sickness, with special reference to prognosis and treatment. *Trans. R. Soc. trop. Med. Hyg.*, XXVII., 67.

Dangerfield, W. G., Gaunt, W. E., and Wormall, A., 1938, Studies on Bayer 205 (Germanin) and Antrypol. I. The determination of small amounts of Bayer 205 (and Antrypol). II. The persistence of Bayer 205 in the blood-stream after injection into animals. *Biochem. J.*, XXXII., 59.

De Marqueissac, H., 1933, Emploi de la médication mixte conjuguée : 205 Bayer- 309 Fourneau + tryparsamide dans le traitement des réinfestations sanguines ou des échècs cliniques constatés chez les malades atteints de trypanosomiase à virus gambiense. *Bull. Soc. Path. exot.*, XXVI., 1051.

Fairbairn, H., 1934, Lange's colloidal gold reaction and the estimation of total proteins in the cerebrospinal fluid of Rhodesian sleeping sickness, and their significance in prognosis. *Trans. R. Soc. trop. Med. Hyg.*, XXVII., 471.

Fourneau, E., 1924, Répresentation cinématographique d'une synthèse chimique (série du " 205 " Bayer). *Bull. Acad. Méd., Paris*, XCI., 458.

Lang, K., 1931, Über das Verhalten des Germanins im Organismus. *Arch. exp. Path. Pharmak.*, CLX., 560.

Lester, H. M. O., 1935, Report of the Tsetse Investigation and Sleeping Sickness Service. *Ann. Rep. Med. Services, Nigeria.* Appendix B, p. 71.

Maclean, G., 1929, Notes on treatment of fifty-two cases of Rhodesian trypanosomiasis with Bayer 205 and tryparsamide. *Ann. trop. Med. Parasit.*, XXIII., 337.

Maclean, G., and Fairbairn, H., 1932, Treatment of Rhodesian sleeping sickness with Bayer 205 and tryparsamide : observations on 719 cases. *Ibid.*, XXVI., 157.

MILLOUS, 1936, Le traitement de la maladie du sommeil au Cameroun. *Ann. Méd. Pharm. colon.*, XXXIV., 966.

SCHLOSSBERGER, H., 1928, Chemotherapie der Infektionskrankheiten in "Handbuch der pathogenen Mikro-organismen" edited by Kolle, Kraus and Uhlenhuth, Berlin and Jena.

STEPPUHN, O., and UTKINA-LJUBOWZEWA, 1924, Eine quantitative Methode zur Bestimmung von "Bayer 205" in Serum, Harn und Gewebe. *Klin. Wschr.*, III., 154.

TRILLAT, A., 1936, Guérison d'une trypanosomiase expérimentale chez la souris par inhalation de vapeurs de moranyl. *Bull. Acad. Méd. Paris*, CXVI., 75.

ZSCHUCKE, J., 1936, Untersuchungen über eine Heilwirkung des "B. 205" auf die Schlafkrankheit des Menschen bei peroraler Verabreichung. *Arch. Schiffs- u. Tropenhyg.*, XL., 449.

QUINOLINE DERIVATIVES

(1) Anil- and Styryl-Quinoline Derivatives

Derivatives of anil- and styryl-quinolines were introduced in the treatment of trypanosomiasis by Browning, Cohen, Ellingworth and Gulbransen (1926 and 1929). The existence of trypanocidal activity in these compounds appears to depend on the presence in each of the nuclei of basic groups or at any rate of acylamino groups. The most marked trypanocidal action was exerted by substances which contain a free basic group in one nucleus and an acylamino group in the other. This was especially noticeable in the styryl series which were, as a rule, much more effective than the anils as trypanocidal agents. The two most active compounds prepared were 2(*p*-acetylaminostyryl)-6-dimethylaminoquinoline methochloride and 2(*p*-aminostyryl)-6-acetylaminoquinoline methochloride. Further analogues of these anil and styryl derivatives have been examined by Browning, Cohen, Cooper and Gulbransen (1932). Compounds endowed with trypanocidal activity are 2(*p*-aminostyryl)-6-acetyllactylamino-quinoline methochloride, 2(*p*-aminostyryl)-6-lactylaminoquinoline methochloride and 2(*p*-aminostyryl)-6-glycerylaminoquinoline methochloride, though on testing a series of doses the range of action appeared to be less than in the compound 2(*p*-aminostyryl)-6-acetylaminoquinoline methochloride of which they are analogues. 2(*p*-Lactylaminostyryl)-6-aminoquinoline methochloride had a wide range and by acetylation of the amino group in the quinoline nucleus trypanocidal activity was not greatly altered. Browning, Cohen, Ellingworth and Gulbransen (1931) have also studied the trypanocidal activity of a number of derivatives of benzthiazole in which the pyridine ring of the quinoline group is replaced by a five-membered ring containing nitrogen and sulphur. Both anil and styrylbenzthiazole derivatives exhibited trypanocidal action when the appropriate groups were present in the respective nuclei, when, that is to say, as in the quinoline series, one nucleus contained a basic group, the other an acetylamino group. As in the quinoline series, compounds containing the following constituent groups were possessed of trypanocidal action :—

Styryl No.	Group in benzene nucleus	Group in benzthiazole nucleus
332	NH_2	$NH.COCH_3$
349	$NH.CH_3$	$NH.COCH_3$
330	$N(CH_3)_2$	$NH.COCH_3$
368	$NH.COCH_3$	NH_2
333	$NH.COCH_3$	$NH_2.COCH_3$
377	$NH.COCH_3$	$N(CH_3)_2$

In the anilbenzthiazole group some action was observed in all the compounds prepared except with 2(*p*-dimethylaminoanil) benzthiazole methochloride but the only compound producing a cure was 2(*p*-methylaminoanil) acetylaminobenzthiazole methosulphate.

A further series of anil and styryl derivatives of 4-aminoquinaldine have been investigated for trypanocidal action by Ashley, Browning, Cohen and Gulbransen (1933). In these compounds the substituent group in the quinoline nucleus is situated in the 4-position and consists of a primary, tertiary or acetylated amino group. 2(*p*-Dimethylaminostyryl)-4-acetylaminoquinoline methochloride was without action, whereas the 6-analogue, although only slightly less toxic for the mammalian host, is an effective trypanocidal agent. 2(*p*-Dimethylaminoanil)-4-acetylaminoquinoline methochloride occasionally produced a cure, while the 6-analogue, which is three times more toxic, has only a slight trypanocidal action.

A further series in which the substituent groups in the quinoline nucleus, either primary, tertiary or acetylated *para*-aminobenzoylamino, are in the 6-position has been investigated by Browning, Cohen, Cooper, Ellingworth and Gulbransen (1933). In the anil derivatives there was some trypanocidal action but no cure was produced. Of the styryl compounds those with a dimethylamino group in the benzene nucleus were without trypanocidal action but others were active. Thus 2(*p*-acetylaminostyryl)-6(*p*-aminobenzoylamino) quinoline methoacetate had a chemotherapeutic index of 1 : 240 : 2(*p*-acetylaminostyryl)-6(*p*-dimethylaminobenzoylaminoquinoline methoacetate) did not act in so wide a range of dosage; certain others were rather irregular in their action.

(2) Surfen C

Of a large number of derivatives of 4-aminoquinoline, one compound, bis-2-methyl-4-aminoquinolyl-6-melamine, " Surfen C " (Iensch, 1937) has been used in the treatment of trypanosome infections. Surfen C must be distinguished from " Surfen," bis-2-methyl-4-aminoquinolyl-6-carbamide, which has recently been introduced as a non-staining antiseptic and is claimed to be more active in living tissues than *in vitro* (von Schürer and Homma, 1937). Although pathogenic trypanosomes isolated from man are cured when maintained in mice the same strains in human beings are not so readily affected and, in addition, surfen C has a toxic action on the kidney. In mice, infections due to *T. brucei* and *T. congolense* are readily cured but, according to Lester (1935), in Nigeria three doses at weekly intervals of 10 mgm. per kilogram of body weight do not prevent relapses in sheep infected with *T. congolense* and *T. vivax*, although the therapeutic results are better than with tartar emetic. In Nigeria also cattle infected with these trypanosomes are not cured by three intramuscular injections of 10 mgm. per kilogram of body weight, as was the case in Uganda with cattle infected by *T brucei* and *T congolense*. Six months later, however, all the Nigerian cattle treated by surfen C were alive, while only 6·2 per cent. of controls survived for the same period. Evans (1936) found that in cattle infected with *T. congolense* a single dose of surfen C was equal in effect to five injections of antimosan. Stewart (1937) on the Gold Coast found that surfen C was of little value in cattle, but curative in canine trypanosomiasis.

In Northern Rhodesia the results in cattle were disastrous : some animals died within fifteen minutes of the injection with froth and blood running from the nostrils and at post mortem hæmorrhages were present in the lungs (Le Roux, 1937).

In horses infected in the Sudan with *T. congolense*, Bennett

(1936) found that almost all animals could be cured with surfen C though the amount of the drug necessary to ensure a cure varied. Some horses are cured by a single dose of 1 gm. per 100 kilograms of body weight. Horses should not be given surfen C intravenously, while on subcutaneous administration large swellings develop at the point of injection: occasionally the same trouble follows intramuscular administration. No generalised toxic reactions were seen in horses.

A quinoline derivative, "Bayer 7602 (Ac.)," in which the two aminomethylquinoline groups are joined by a diallylmalonyl group, has been given to a three months old child infected with *T. cruzi*. The acute symptoms were cured by four injections of a 3 per cent. watery solution of the drug, 3·75 c.c. in all, but the patient relapsed (Mazza, Cossio and Zuccardi, 1937).

References

ASHLEY, J. N., BROWNING, C. H., COHEN, J. B., and GULBRANSEN, R., 1933, The antiseptic and trypanocidal properties of some anil and styryl derivatives of 4-aminoquinaldine. *Proc. R. Soc.*, B., CXIII., 293.

BENNETT, S. C. J., 1936, The treatment of equine *Trypanosoma congolense* infections with surfen C (Bayer). *J. comp. Path.*, XLIX., 151.

BROWNING, C. H., COHEN, J. B., COOPER, K. E., ELLINGWORTH, S., and GULBRANSEN, R., 1933, The antiseptic and trypanocidal action of some benzoylaminoquinoline anil and styryl compounds. *Proc. R. Soc.*, B. CXIII., 300.

BROWNING, C. H., COHEN, J. B., COOPER, K. E., and GULBRANSEN, R., 1932, The trypanocidal action of some derivatives of anil and styryl quinolines. II. *Proc. R. Soc.*, B. CX., 372.

BROWNING, C. H., COHEN, J. B., ELLINGWORTH, S., and GULBRANSEN, R., 1926, Aminostyrylquinoline and aminoanilquinoline compounds. A new group of trypanocidal agents. *J. Path. Bact.*, XXIX., 317.

BROWNING, C. H., COHEN, J. B., ELLINGWORTH, S. GULBRANSEN, R., 1929, The trypanocidal action of some derivatives of anil and styryl quinoline. *Proc. R. Soc.*, B. CV., 99.

BROWNING, C. H., COHEN, J. B., ELLINGWORTH, S., and GULBRANSEN, R., 1931, The antiseptic and trypanocidal action of certain styryl and anil benzthiazole derivatives. *Proc. R. Soc.*, B. CVIII., 119.

EVANS, J. T. R., 1936, *Trypanosoma congolense* infection in cattle: treatment by antimosan and surfen "C." *J. comp. Path.*, XLIX., 160.

IENSCH, H., 1937, Neue Chemotherapeutika der 4-Amino-chinolin-Reihe. *Angewandte Chemie*, L., 891.

LESTER, H. M. O., 1935, Report of the Tsetse Investigation, Nigeria. Rep. Med. Hlth. Services for the year 1934. Appendix B, p. 69.

LE ROUX, P. L., 1937, Trypanosomiasis. Ann. Rept. for the year 1936. Vet. Dept. Govt. of Northern Rhodesia. Appendix C, p. 64.

MAZZA, S., COSSIO, R., and ZUCCARDI, E., 1937, Primer caso agudo grave de enfermedad de Chagas comprobado en Tucumán y su tratamiento con Bayer 7602 (Ac.). *Universidad Buenos Aires: Misión de Estudios de Patologia regional Argentina. Jujuy.* Publicación No. 32, p. 3.

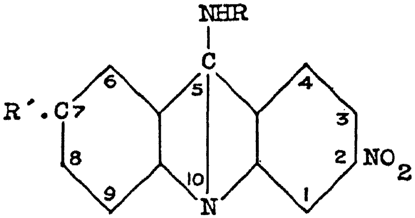
STEWART, J. L., 1937, Trypanosomiasis. Rept. Dept. Animal Hlth. for the year 1936–37. Gold Coast Colony, p. 15.

VON SCHÜRER, F., and HOMMA, H., 1937, "Surfen" ein neues Oberflächen- und Tiefenantiseptikum. *Münch. med. Wschr.*, **LXXXIV.**, 1524.

ACRIDINE DERIVATIVES

Acriflavine, which has long been known to possess trypanocidal properties, is, according to the Addendum (1936) to the British Pharmacopœia, a mixture of 2 : 8-diamino-10-methylacridinum chloride and 2 : 8-diaminoacridine and contains approximately one-third of its weight in the form of the latter compound.

Schnitzer and Silberstein (1929) have found that in the case of the 6-nitro-9-aminoacridines the presence of the nitro group in position 6 enhances trypanocidal action and a further favourable effect is brought about by the substitution of a doubly alkylated amino group for a hydrogen atom in the amino group in position 5.



In this formula the NHR in position 5 is such a substituent group as the dimethylaminooxypropylamino group $NH.CH_2.CHOH.CH_2.NMe_2$, whilst R′ in position 7 is an alkyloxy group such as methoxyl (OMe). So far as trypanocidal action is concerned the doubly alkylated basic side chain (R) may either replace a hydrogen atom in the amino group at position 5 or it may be directly attached to the carbon atom in position 6. Within a group of substances in which R has the same composition and position, modification of trypanocidal action could be brought about in most cases by variation of R′ (position 7); thus a 2-ethoxy compound is usually less active than a 2-methoxy compound and the latter less active than one in which there is no side-chain in position 7.

Toxicity and therapeutic effect do not always run on parallel lines in these compounds. In some groups the most toxic members are also the most effective therapeutically, in others only the possibility of large dosage allows the exhibition of any therapeutic activity. By variation of the side-chains within the limits described, Schnitzer and Silberstein believe, however, that it is possible to obtain a favourable adjustment between toxic action and therapeutic efficiency. Thus compound 3043a with a glycyldiethylaminoethylamino group, probably $CH_2.NH_2.CO.CH_2.CH_2.NEt.CH_2.CH_2.NH$ in position 5 and an ethoxyl group (OC_2H_5) in position 7, has a chemotherapeutic index of from 1 : 20 to 1 : 50 and is, moreover, one of the few instances where an ethoxyl is more efficient than a methoxyl group in position 7.

The action of a number of pyrrole dyes has been tested by Fischl (1935) on *T. brucei* infections in mice. Pyrrol blue, pyrrol red and phthalocyanin had no trypanocidal action but the perchlorate of a red dye obtained from *Bacillus prodigiosus* and termed prodigiosin was found to have a temporary curative action.

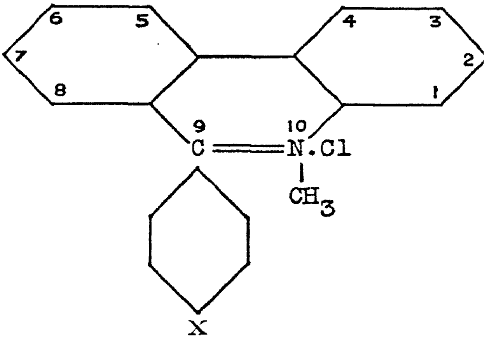
References

FISCHL, V., 1935, Chemotherapeutische Prüfung einiger Pyrrolfarbstoffe. *Z. ImmunForsch.*, LXXXV., 77.

SCHNITZER, R., and SILBERSTEIN, W., 1929, Über neue trypanocide Acridinfarbstoffe. Untersuchungen an homologen Reihen von 6-Nitro-9-Aminoacridinen., *Z. Hyg. InfektKr.*, CIX., 519.

PHENANTHRIDINIUM COMPOUNDS

Two of a series of phenanthridine and phenanthridinium compounds were found by Browning, Morgan, Robb and Walls (1938) to possess curative action in mice experimentally infected with trypanosomes. These, in relation to the formula, are :



A. 7 - amino - 9(*p* - aminophenyl) - 10 - methylphenanthridium chloride (NH_2 groups at positions 7 and X)

B. 3(acetylamino) - 9(*p*-acetylaminophenyl) - 10 - methylphenanthridinium chloride ($NH.COCH_3$ groups at positions 3 and X).

Both of these substances in doses approaching the maximum tolerated (one subcutaneous injection of 1 mgm. of A or 2·5 to 5·0 mgm. of B for a mouse of 20 gm.) are curative for mice infected with *T. brucei* when injected twenty-four hours after inoculation. Compound A was found also to have a curative action on *T. congolense* infections in mice, while compound B, which is without effect on this trypanosome, has a marked therapeutic action on *Spirillum minus* in mice, though even the largest doses do not lead to cure. Both compounds act on a strain of *T. brucei*, which is completely resistant to the largest tolerated doses of arsacetin. These phenanthridinium compounds constitute a new type of trypanocidal agent.

Reference

BROWNING, C. H., MORGAN, G. T., ROBB, J.U.M., and WALLS, L. P., 1938, The trypanocidal action of certain phenanthridinium compounds. *J. Path. Bact.*, **XLVI.**, 203.

GUANIDINE DERIVATIVES

Since in experimental trypanosome infections the blood sugar is decreased, a number of observers have attempted to determine whether interference with the normal carbohydrate metabolism affects in any way the multiplication of trypanosomes. *In vitro* Christophers and Fulton (1938) showed that in the presence of glucose, trypanosomes take up large quantities of oxygen. Unlike malaria parasites trypanosomes rapidly die in the absence of glucose, while even in the presence of glucose respiration is reduced if the *p*H becomes acid. Poindexter (1935) showed that injections of insulin *in vivo* decrease the rate of multiplication of trypanosomes and prolong the life of the host, while insulin shock is more readily produced than in the normal animal. Von Jancsó and von Jancsó (1935) and Schern and Artagaveytia-Allende (1935 and 1936) also showed that synthalin and synthalin B had a therapeutic effect on *T. brucei* infections in mice and *T. equiperdum* infections in rats, the morphological changes in the trypanosomes being similar to those produced by germanin. It was therefore suggested that synthalin acts directly on the trypanosomes. This view was confirmed by the experiments of Lourie and Yorke (1937) who found that *in vitro* at 37° C. synthalin in a dilution of 1 : 256,000,000 exerts a trypanocidal action within twenty-four hours. The action of synthalin thus could not be directly due to hypoglycæmia. P. Browning (1938) obtained similar results on *T. brucei* and *T. congolense*. Synthalin acted on an arsacetin-resistant strain of *T. brucei* equally as well as on a normal strain. The trypanocidal action of a number of guanidine derivatives was therefore studied by King, Lourie and Yorke (1937) in view of the fact that the chemical constitution of synthalin is essentially different from that of all other known trypanocidal substances. It was found that in a series of homologues of synthalin trypanocidal action *in vitro* rises as the number of methylene groups in the alkylene chain increases till when the number of methylene groups is 10 the action *in vitro* is comparable to that of the tervalent arsenicals. Thus *in vitro* decamethylene diguanidine dihydrochloride (synthalin), dodecamethylene diguanidine dihydrochloride (synthalin B) and tetradecamethylene diguanidine dihydrochloride are all trypanocidal in a dilution of 1 : 256,000,000. Unfortunately with lengthening of the alkylene chain the toxicity to animals also increases. *In vivo* therapeutic activity begins with the octamethylene member of the series, maximum tolerated doses of which clear the blood of infected mice after a delay of some days. With synthalin a dose of 0·05 gm., approximately a third of the toxic dose, clears the blood of infected mice for five or six days. Alkylguanidines exhibit trypanocidal action *in vitro* but less than the corresponding diguanidines and *in vivo* their action is nil. Certain simple cyclic guanidines also have a very weak action *in vitro*: this weak action is especially noteworthy in the case of diguanylspermine which differs from synthalin only in the breaking of the chain of ten methylene groups by two imino groups. The guanidine grouping $NH : C(NH_2)NH—$ is not, however, essential for trypanocidal activity, as is shown by the result of an examination of a series of alkylene-di*iso*-thioureas in which the connecting link between the guanyl grouping $NH : C(NH_2)—$ and the carbon (alkylene) chain is not an imino group —NH— but a sulphur atom, *e.g.*, $NH : C(NH_2)—S—NH_2—$. Here also *in vitro* trypanocidal action increases as the series is ascended. High trypanocidal activity is present in the alkylene diamidines in which two guanyl groups are attached directly to the ends of the methylene chain without the intervention of other groups or atoms. Again, maximum activity is reached as the series is ascended, undecane diamidine exhibiting pronounced trypanocidal activity *in vitro* and a curative action in both infected mice and rabbits. Activity is decreased in the amidine series with members containing more than eleven carbon atoms.

When the guanyl group is replaced by an amino group there is still a noticeable trypanocidal action *in vitro* which seems to reach its maximum in the hexadecylamine member of the series. In the alkylene-diamines a still greater activity is observed *in vitro* and possibly the maximum for the series has not yet been reached.

Since quarternary ammonium compounds possess only slight trypanocidal activity and cannot give rise to free bases by hydrolysis it is possible that the trypanocidal activity of the guanidines, amidines, amines and *iso*thioureas is a property of the free bases since all these groups are strong bases forming neutral salts.

Therapeutically in mice infected with *T. rhodesiense* undecane diamidine appears to be superior to decamethylene diguanidine (synthalin), for while the maximum tolerated dose of synthalin is from 0·075 to 0·1 mgm. per 20 gm. mouse, the corresponding dose of undecane diamidine is at least 0·25 mgm. The minimum lethal dose of undecane diamidine is about 0·5 mgm. as compared with 0·15 to 0·25 mgm. for synthalin. In the rabbit the maximum tolerated dose of undecane diamidine (10 mgm. per kilogram) causes a transient hyperglycæmia with nitrogen retention. Lethal doses produce a fatal hyperglycæmia with or without initial hyperglycæmia : histological changes are found in the liver and kidneys. In man 150 mgm. per day given by mouth on three consecutive days does not affect the blood sugar level but brings about nitrogen retention. Nausea is a prominent symptom (Devine, 1938). Undecane diamidine has little curative action on mice infected with *T. congolense*, but when given in large doses on the day of inoculation and on the nine following days it exhibits

THE TRYPANOCIDAL ACTIVITY OF THE ALKYLENE DIAMIDINES

Compound	Trypanocidal titre *in vitro*	Therapeutic effect	Toxicity in gm. per 20 gm. mouse
Octamethylenediamidine 2HCl	1 : 8,000,000	very slight	1·0
N-Decane 1 : 10-diamidine 2HCl	1 : 64,000,000	curative	0·5
N-Undecane 1 : 11-diamidine 2HCl	1 : 256,000,000	,,	0·5
N-Dodecane 1 : 12-diamidine 2HCl	1 : 64,000,000	,,	0·5

a pronounced prophylactic action. It has no action on *T. cruzi* infections in mice.

A number of aromatic amidine and guanidine compounds were found by King, Lourie and Yorke (1938) to show a pronounced trypanocidal action *in vitro*, and with three of them, *pp'*-diguanidino-diphenyl-methane, *pp'*-diamidino-diphenyl-methane and 2 : 7-naphthalene diamidine it was possible to cure mice infected with *T. rhodesiense*. The discovery that trypanocidal activity resides in compounds of this type opens up a wide new field for experimental research which may possibly be extended to the chemotherapy of malaria, since Glyn-Hughes, Lourie and Yorke (1938) have found that undecane diamidine has a very definite but temporary action in induced benign tertian and quartan malaria in man.

References

Browning, P., 1938, The chemotherapeutic action of synthalin in experimental infections with *T. brucei* and *T. congolense*. *J. Path. Bact.*, XLVI., 323.

Christophers, S. R., and Fulton, J. D., 1938, Observations on the respiratory metabolism of malaria parasites and trypanosomes. *Ann. trop. Med. Parasit.*, XXXII., 43.

Devine, J., 1938, Studies in chemotherapy, XVIII. Changes in the blood and urine produced by administration of undecane diamidine. *Ibid.*, XXXII., 163.

Glyn-Hughes, F., Lourie, E. M., and Yorke, W., 1938, Studies in chemotherapy, XVII. Action of undecane diamidine in malaria. *Ibid.*, XXXII., 103.

Jancsó, N. von, and Jancsó, H. von, 1935, Chemotherapeutische Wirkung und Kohlehydratstoffwechsel. Die Heilwirkung von Guanidinderivaten auf die Trypanosomeninfektion. *Z. ImmunForsch.*, LXXXVI., 1.

King, H., Lourie, E. M., and Yorke, W., 1937, New trypanocidal substances. *Lancet, ii*, 1360.

King, H., Lourie, E. M., and Yorke, W., 1938, Studies in chemotherapy, XIX. Further report on new trypanocidal substances. *Ann. trop. Med. Parasit.*, XXXII., 177.

Lourie, E. M., and Yorke, W., 1937, Studies in chemotherapy, XVI. The trypanocidal action of synthalin. *Ann. trop. Med. Parasit.*, XXXI., 435.

Poindexter, H. A., 1935, Further observations on the relation of certain carbohydrates to *Trypanosoma equiperdum* metabolism. *J. Parasit.*, XXI., 292.

Schern, K., and Artagaveytia-Allende, R., 1935, La terapie de la tripanosomosis y espirilosis. *Arch. Soc. Biol., Montevideo*, VI., 244.

Schern, K., and Artagaveytia-Allende, R., 1936, Zur glykopriven Therapie und Prophylaxie mit sowohl toxisch als auch atoxisch wirkenden Substanzen bei der experimentellen Trypanosomen-und Treponemeninfektion. *Z. ImmunForsch.*, LXXXIX., 21.

DRUG RESISTANCE

Drug resistance was first discovered by Franke and Roehl (1907) who, working in Ehrlich's laboratory, noted that when parafuchsin was fed to mice infected with *T. brucei* the parasites disappeared from the peripheral blood ; after a week or two the parasites reappeared and further administration of the drug caused them to disappear again. The process, however, could not be repeated indefinitely as after a time the parafuchsin produced less and less effect until finally a period was reached when the drug entirely failed to influence the parasites. On transference of the parasites to normal mice they were found to be still uninfluenced by parafuchsin and it became evident that they had in fact acquired a heightened resistance to the drug. This fundamental observation was quickly followed by the discovery that strains of trypanosomes resistant to other drugs could be developed. In fact there are now few, if any, compounds with a chemotherapeutic action on trypanosomes which have not been shown to be capable of giving rise to drug-resistant strains.

The principal substances capable of inducing resistant strains include :—

(1) **Aniline dyes :** parafuchsin, acriflavine (trypaflavine), dichloroparafuchsin (tryparosan), etc.

(2) **Aromatic compounds of arsenic :** atoxyl, arsacetin, tryparsamide, reduced tryparsamide thioglycollate, halarsol, orsanine, 3-amino-4-hydroxyphenylarsonic acid (Fourneau 189), 3-hydroxymethyl-4-aminophenylarsonic acid (Fourneau 683) and 2-hydroxy-4-hydroxymethylphenylarsonic acid (Fourneau 722), neoarsphenamine, arsenophenylglycine, pyridone-arsonic derivatives (Schlossberger and Schüffner, 1934).

(3) **Aromatic compounds of antimony :** stibenyl, fuadin.

(4) **Non-aromatic compounds of antimony :** tartar emetic.

(5) **Bayer 205.**

(6) **Styryl quinoline derivatives :** 2(*p*-acetylaminostyryl-6-dimethylaminoquinoline methochloride and 2-(*p*-acetyl)-

aminostyryl)-6-dimethylaminoquinoline methochloride (Browning, Cohen, Ellingworth and Gulbransen, 1929).

(7) **Surfen C** (Schilling *et al.*, 1936).

(8) **Guanidine compounds :** synthalin (decamethylene diguanidine hydrochloride) *cf.* Schern and Artagaveytia-Allende, 1936, and Browning, 1938, and N-undecane 1 : 11-diamidine (Lourie and Yorke, 1938).

(9) **Serum.**

The ease with which resistant strains are produced shows, however, much variation. Yorke, Murgatroyd and Hawking (1932) found that with atoxyl, arsacetin, tryparsamide, reduced tryparsamide thioglycollate, stibenyl and acriflavine highly resistant strains are produced within four to eight weeks. After a single massive injection of tryparsamide in a cat infected with *T. annamense* the relapse strain which appeared in the blood-stream three months later was found by Launoy (1937) to be wholly resistant to tryparsamide and acriflavine. Bovet and Montézin (1937), on the other hand, working with an old laboratory strain of *T. brucei*, failed to produce any recognisable degree of resistance on the part of the trypanosome either to atoxyl or orsanine with a single dose of atoxyl (1 mgm. per 20 gm. mouse). A single dose of orsanine, tryparsamide or 3-amino-4-hydroxyphenylarsonic acid also failed to produce resistance. A single dose of 3-hydroxymethyl-4-aminophenylarsonic acid (10 mgm. per 20 gm. mouse) produced great resistance both to itself and to orsanine, as also did a single dose of 2-hydroxy-4-hydroxymethylphenylarsonic acid.

Naito and Oka (1936) found that maximum resistance in a strain of *T. brucei* was reached in the case of orsanine after thirteen passages, but in the case of neoarsphenamine and silver neoarsphenamine not until the fiftieth passage in mice.

Yorke and Murgatroyd (1930) have pointed out the importance of the substituted phenyl radical for the presence and degree of resistance. Hawking (1937) has drawn attention to the importance of the side-chains attached to the phenylarsenoxide nucleus in determining the presence and degree of resistance to these compounds. A similar influence of the side-chain can be traced upon the chemotherapeutic index of the various compounds :

the side-chain tends to diminish the toxicity while leaving the trypanocidal activity for normal trypanosomes relatively unchanged (*cf.* Table, page 236). Hawking suggests that the phenylarsenoxide nucleus combines with "receptors" of the normal trypanosome but that such combination with the "receptors" of the resistant trypanosome is prevented by the presence and nature of the side-chain. It is possible that when the receptors of the trypanosome are modified in the development of drug resistance, they tend to approximate to those of the host.

The production of halarsol- and neoarsphenamine-fast strains requires a period of about three months, the arsenophenylglycine-fast strain develops still more slowly and the Bayer 205-fast strain of *T. rhodesiense* does not usually attain its maximum degree of resistance till after more than twelve months. Leupold (1925), who used a strain of *T. brucei*, also found that maximum resistance to germanin was not obtained till after more than one hundred passages. On the other hand, von Jancsó and von Jancsó (1935 [1 & 2]) showed that a very considerable degree of germanin-fastness could be induced after twelve treatments in mice, provided the activity of the reticulo-endothelial system was eliminated, first by splenectomy two to four hours before treatment, and secondly by the intravenous injection of colloidal copper three to four hours after the injection of germanin.

Strains to become resistant to undecane diamidine, although they can be produced both in the mouse and rabbit and *in vitro*, require exposure for some considerable time (Lourie and York, 1938).

It is suggested that trypanosomes possess a capacity for adapting themselves to germanin but in the normal animal this tendency is counteracted by the reticulo-endothelial system. Yorke, Murgatroyd and Hawking (1932 [1]) draw attention to the fact that it is not easy to produce a strain resistant to tartar emetic: nevertheless an atoxyl-fast strain in mice may be made resistant to tartar emetic by two or three doses. This may have an important bearing on the treatment of trypanosome infections in man with atoxyl and tartar emetic. It will thus be seen that different compounds, even when closely related chemically, exhibit very different aptitudes for provoking drug resistance. In so far as the aromatic arsenicals are concerned the production of resistance is in no way proportional to the amount of arsenic in the compound.

The more important strains of drug-fast trypanosomes may be arranged in the following groups :—

(*a*) Strongly resistant to the aromatic compounds of arsenic (except arsenophenylglycine) and of antimony : slightly resistant to arsenophenylglycine : sensitive to tartar emetic, Bayer 205, and certain phenanthridinium compounds (*cf.* Browning *et al.*, 1938), viz., atoxyl-fast, arsacetin-fast, tryparsamide-fast, reduced tryparsamide-fast, orsanine-fast, halarsol-fast, neoarsphenamine-fast and acriflavine-fast strains.

(*b*) Resistant to arsenophenylglycine : moderately resistant to the other aromatic arsenicals and antimonials, sensitive to tartar emetic and to Bayer 205, viz., arsenophenylglycine-fast strains.

(*c*) Similar to Group (*a*) but resistant to tartar emetic, viz., tartar emetic-fast strain.

(*d*) Strongly resistant to Bayer 205 : sensitive to all other drugs, viz., Bayer 205-fast strain.

(*e*) Resistant to amidine and guanidine compounds : sensitive to aromatic compounds of arsenic and antimony, acriflavine and Bayer 205.

From these facts, as Yorke and his colleagues (1932) have pointed out, it is possible to make the following generalisations :—

(1) Resistance to an aromatic arsenical or antimonial compound implies resistance to other aromatic arsenicals and antimonials (*cf.* also Dubois, 1936), but this does not necessarily mean that a strain made resistant to one aromatic arsenical is identical with that made resistant to another. This is seen in the differences in degree of resistance exhibited by a tryparsamide-fast strain, an arsenophenylglycine strain or one made resistant to the monosodium salt of pyridone-3-arsonic acid (*cf.* Schlossberger and Schüffner, 1934).

(2) A strain made resistant to an aromatic arsenical is sensitive to tartar emetic, whereas a strain made resistant to an aromatic antimonial is resistant to tartar emetic. This is probably due to the fact that stibenyl, like the other aromatic antimonial compounds, easily undergoes a certain amount of decomposition when introduced into the animal body.

THE STABILITY OF DRUG RESISTANCE IN TRYPANOSOMES

In considering the stability of drug resistance in trypanosomes three questions require discussion :—

(1) The duration of the resistance.

(2) The persistance of the resistance when the trypanosomes are passaged through *Glossina.*

(3) The effect of passage from one animal species to another.

(1) The duration of drug resistance to aromatic compounds of arsenic to antimony and to acriflavine in trypanosomes was shown by Murgatroyd and Yorke (1937) to persist indefinitely. A strain of *T. rhodesiense* maintained its resistant character to atoxyl for a period of seven and a half years, during which the strain had passed through a series of nine hundred mice. Strains of *T. rhodesiense,* made resistant to tryparsamide and to acriflavine respectively, behaved similarly while a strain of *T. brucei* also maintained its resistant character unimpaired for a period of four years. Browning (1908), however, noted that an atoxyl-resistant strain had lost this character in its eighty-ninth passage. Robertson (1929) also found a slow loss of resistance to acriflavine on the part of *Bodo.* In addition resistance to Bayer 205 is gradually lost, for a strain of *T. rhodesiense* made resistant to Bayer 205 had within a year lost at least 90 per cent. of its resistance and within four years had lost all trace of resistance. Attempts to influence or break down arsenic resistance by exposure to heat were unsuccessful (Janssens, 1936).

(2) The question whether a drug-resistant strain is still drug-resistant after cyclical development in its insect host has been studied by Werbitzki (1910), Gonder (1911), Reichenow and Regendanz (1927) and Duke (1927), whose experiments gave somewhat indefinite results possibly because their strains were scarcely more resistant than the parent strains. More recently Yorke, Murgatroyd and Hawking (1933 [1 & 2]) and Murgatroyd and Yorke (1937) have reinvestigated the question and have shown that a strain of *T. brucei* made resistant to tryparsamide could be transmitted four times through *Glossina morsitans* during a period of four years without in any way losing its resistance. Passage

of this tryparsamide-fast strain through *G. palpalis* was also obtained, though less readily (*cf.* Murgatroyd, Yorke and Corson, 1937). Resistance to Bayer 205 survived at least one passage through *G. morsitans*. Lester (1932) also showed that a strain of *T. brucei* rendered serum-fast retained this character after at least one cyclical transmission through *G. tachinoides*.

The retention of drug resistance after passage through *Glossina* is obviously of great practical importance, for, as a result of insufficient treatment, strains of trypanosomes resistant to the aromatic arsenicals have undoubtedly been produced in man. If such arsenic-resistant strains are taken up by tsetse flies and transmitted to new human hosts these unfortunate individuals will not be cured by the administration of therapeutic doses of the aromatic arsenicals. Barlovatz (1933), for instance, has reported cases from the Belgian Congo in which the trypanosomes were, from the beginning, resistant to tryparsamide; as a result the patients failed to react to arsenical treatment.

(3) The effect on drug resistance of passage from one animal species to another was first investigated by Mesnil and Brimont (1908) who came to the conclusion that a strain of trypanosomes made resistant to atoxyl in the mouse may lose this resistance when transferred to other hosts. This suggestion was supported by the work of Breinl and Nierenstein (1908) and others but has been entirely contradicted by Yorke and Hawking (1932) who showed that a strain of *T. rhodesiense*, made resistant to atoxyl in the mouse, continued to manifest its full resistance when transferred to and passaged through rats or rabbits: a strain made resistant to tryparsamide in rabbits also manifested complete resistance when subsequently transferred to mice. *In vitro* experiments also yielded results which indicated that the character of drug resistance is not modified by transference of the trypanosome from one host to another.

Citron (1931) claimed to have destroyed the resistance of an arsphenamine-fast strain of trypanosomes by prolonged treatment of a series of infected mice with sodium thiosulphate. Yorke, Murgatroyd and Hawking (1932 [2]), however, failed to confirm these results.

THE MECHANISM OF DRUG RESISTANCE IN TRYPANOSOMES

Although drug resistance has been studied for more than thirty years, it is only recently that, largely as a result of the work of Yorke and his colleagues, the phenomenon has been placed on a secure experimental basis of ascertained fact. The first step in this experimental investigation was the development of a technique whereby suspensions of trypanosomes could be kept alive *in vitro*, in undiminished numbers and with unlowered vitality, for at least twenty-four hours at 37° C. (Yorke, Adams and Murgatroyd, 1929). In 1909, Ehrlich had observed that trypanosomes resistant to dyes remained unstained, and in conformity with this finding, Yorke and Murgatroyd (1930) and Yorke, Murgatroyd and Hawking (1931), demonstrated that *in vitro* trypanosomes removed reduced tryparsamide from the normal medium, while resistant ones did not. No investigations were made to determine whether removal was due to absorption or neutralisation. In 1932, however, Reiner, Leonard and Chao (1932 [1 & 2]) found that *in vitro* at room temperature normal trypanosomes did absorb appreciable quantities of the tervalent arsenicals, neoarsphenamine, sulpharsphenamine, stabilarsan and 3-amino-4-hydroxyphenyl-arsenoxide, the arsenic being determined chemically: the quinquevalent atoxyl was not absorbed. Dead trypanosomes absorbed much more than living ones while resistant trypanosomes bound rather less than normal ones. The difference was thought not to be sufficiently great to explain the mechanism of drug resistance. Pedlow and Reiner (1935), in reinvestigating the problem at 37° C., found that, *in vitro*, arsenic-resistant trypanosomes fixed the same amount of arsenic as did normal trypanosomes, although *in vivo* they fixed a somewhat smaller amount. It has been suggested (*cf.* Hawking, 1937) that the strain of trypanosome employed by these workers was possibly not maximally resistant, rather strong concentrations of the drug were employed and in the majority of the experiments the drug used was neoarsphenamine, a compound which appears to be somewhat unsatisfactory for work of this kind.

Fischl and Singer (1934 [1]) found that if normal trypanosomes are exposed *in vivo* to atoxyl, tryparsamide and neoarsphenamine they take up measurable quantities of arsenic : with the naturally resistant *T. lewisi* an appreciable quantity of arsenic was bound after exposure to arsenophenylglycine, a smaller amount after exposure to atoxyl and arsphenamine and none after exposure to neoarsphenamine, solusalvarsan and sodium arsenite (Singer and Fischl, 1935). Similarly, normal trypanosomes absorb gold when exposed *in vivo* to 3 : 3′-di-auromercapto-4 : 4′-disulpho-*s*-diphenylcarbamide "Sulphoharnstoff" (Fischl, Kotrba and Singer, 1934) : absorption occurred also with *T. lewisi*, although the gold compounds used were not trypanocidal. Hawking (1937), found that normal trypanosomes absorb all the available drug from suitable concentrations of tervalent compounds such as tryparsamide, halarsol and neoarsphenamine, and with low concentrations of the drug, the trypanosomes suffer no appreciable damage during the process. Absorption occurs very rapidly, being complete at 37° C. in a few minutes, though at 5° C. the process takes a few minutes longer. Living resistant trypanosomes absorb little or none of the drug from similar concentrations of the above compounds, but absorption will occur if stronger concentrations are used or if the trypanosomes are dead. A similar difference between the behaviour of normal and resistant trypanosomes is observed when the parasites are exposed to reduced tryparsamide *in vivo*. Compounds to which the atoxyl-resistant trypanosomes show no resistance, such as phenylarsenoxide, sodium arsenite and tartar emetic, are absorbed to the same extent by normal and resistant organisms. Arsenophenylglycine, which is rather less active on the resistant trypanosomes than on the normal ones, is absorbed in somewhat smaller amounts by the former. The quinquevalent compound, tryparsamide, which is inactive *in vitro* is not absorbed either by normal or atoxyl-resistant trypanosomes.

The action of the dyes of the acridine series, which chemotherapeutically resemble the aromatic arsenical compounds, was first studied by Roehl and Gulbransen (1909) and Gonder (1912), who observed the different behaviour of normal and resistant trypanosomes to vital-staining. Leupold (1925) found that in arsenic-resistant trypanosomes the para-basal body could not be destroyed by exposure to acriflavine, as was the case with normal ones. Von Jancsó (1931 and 1932) demonstrated very conclusively that normal trypanosomes absorb acriflavine, while resistant ones do not, since they are less photosensitive and their blepharoplasts do not take up the dye, an observation confirmed by Jadin (1932), Hasskó (1932), Fischl and Singer (1934 [2] and 1935) and Pedlow and Reiner (1935), as well as by Hawking (1937), who showed that the reaction occurred very rapidly at any rate *in vitro*, since it was complete in a few minutes. Singer and Fischl (1935) have pointed out that when trypanosomes are continuously exposed to non-trypanocidal substances such as atebrin or rivanol, they acquire the power of absorbing at least two and a half times the amount taken up to begin with. The evidence is thus strongly in favour of the view that trypanosomes resistant to aromatic compounds escape injury because they fail to absorb the drug. Similar evidence is available in the case of relapsing fever spirochætes. Fischl and Singer (1934 [1]) and Fischl, Kotrba and Singer (1934) found that normal spirochætes absorb arsenic and gold from neoarsphenamine and solganal respectively but resistant spirochætes fail to absorb either element (Fischl and Singer, 1934 [2]). Feldt (1934) found that both normal and resistant spirochætes absorbed arsenic or gold when exposed to arsphenamine or solganal *in vivo*: in some respects his technique was unsatisfactory.

The reason why resistant trypanosomes and spirochætes absorb drugs less readily than normal organisms is at present unknown, although there is a possibility that the change may be associated with a loss or, more probably, a modification of "receptors."

The increase in resistance acquired by trypanosomes as a result of repeated exposure to the concentration of the drug which first fails to destroy them is however often very considerable. Yorke (1934), for instance, has shown that the concentrations of reduced tryparsamide necessary to kill normal and resistant *T. rhodesiense* are 0·04 and 10 parts per million, respectively, a 250-fold difference.

It is thus obvious that the resistant trypanosomes possess a property which is certainly absent from the normal trypanosomes.

The exact mechanism by which this new character is acquired is still uncertain. On the one hand it may be analogous to the appearance of adaptive enzymes in bacteria, when the bacteria are grown on a substrate containing unusual chemical substances. Adaptive enzymes in bacteria, however, are rapidly lost if and when the substrate returns to normal, while drug resistance is in most cases a comparatively stable character.

It seems more probable that the acquirement of drug resistance is due to the occurrence of mutations in a population subjected to intensive selection over a number of generations. The further question then arises whether the mutant forms arise spontaneously, or as a direct result of environmental change, an example of Lamarckian inheritance. Unfortunately, before this question can be settled it would be essential to know accurately the variation in drug resistance of the descendants of a single trypanosome. If the range of variation of drug fastness of a normal strain to an aromatic arsenical were represented as —10 to + 10, the highest concentration of the drug which just fails to destroy them all would then allow the survival of the trypanosomes with a resistance of + 10. These trypanosomes would then vary in resistance from 0 to 20 and so on till only highly resistant trypanosomes eventually remained. The production of a drug-fast strain of trypanosomes is in fact analogous to the gradual adaptation of free-living organisms to an increase of temperature. Such adaptation occurs in a series of steps which are dependent on the appearance of mutants capable of living at higher temperatures.

References

Barlovatz., A., 1933, Une démonstration statistique de la transmissibilité à l'homme de trypanosomiase résistante au tryponarsyl. *Bull. Soc. Path. exot.*, XXVI., 623.

Bovet, D., and Montézin, G., 1937, Sur l'aptitude remarquable que possèdent différents médicaments arsenicaux à produire des races de trypanosomes résistants. *Ibid.*, XXX., 68.

Breinl, A., and Nierenstein, M., 1908, Weitere Beobachtungen über Atoxylfestigkeit der Trypanosomen. *Dtsch. med. Wschr.*, XXXIV., 1181.

Browning, C. H., 1908, Chemotherapy in trypanosome infections : an experimental study. *J. Path. Bact.*, XII., 166.

BROWNING, C. H., COHEN, J. B., ELLINGWORTH, S., and GULBRANSEN, R., 1929, The trypanocidal action of some derivatives of anil and styryl quinoline. *Proc. roy. Soc.*, B., CV., 99.

BROWNING, C. H., MORGAN, G. T., ROBB, J. V. M., and WALLS, L. P., 1938, The trypanocidal action of certain phenanthridinium compounds. *J. Path. Bact.*, XLVI., 203.

BROWNING, P., 1938, The chemotherapeutic action of synthalin in experimental infections with *T. brucei* and *T. congolense*. *Ibid.*, XLVI., 323.

CITRON, H., 1931, Versuche ueber die Beeinflussung der Salvarsanfestigkeit. *Z. ImmunForsch.*, LXIX., 464.

DOBELL, C., 1912, Some recent work on mutation in micro-organisms. *J. Genet.*, II., 210.

DUBOIS, A., 1936, La résistance des trypanosomes aux arsenicaux aromatiques s'étend à d'autres produits aromatiques non-arsenicaux. *Ann. Soc. belge Méd. trop.* XVI., 173.

DUKE, H. L., 1927, Experiments on the cyclical transmission by *Glossina* palpalis of arsenic-fast strains of polymorphic trypanosomes. *Rep. int. Comm. League of Nations for the Study of Human Trypanosomiasis*, p. 25.

EHRLICH, P., 1909, Ueber Partialfunktionen der Zelle. *Münch. med. Wschr.*, LVI., 217.

FELDT, A., 1934, Ueber den Metallgehalt von Rekurrensspirochäten nach Behandlung infizierter Ratten mit Solganal und Salvarsan. *Zbl. Bakt.* Abt. I. Orig., CXXXI., 137.

FISCHL, V., KOTRBA, J., and SINGER, E., 1934, Der Nachweis von Gold in Spirochäten und Trypanosomen. *Z. Hyg. InfektKr.*, CXVI., 69.

FISCHL, V., and SINGER, E., 1934[1], Gewinnung und Verhalten arzneifester Recurrensspirochäten. *Ibid.*, CXVI., 138.

FISCHL, V., and SINGER, E., 1934[2], Die Wirkungsweise chemotherapeutisch verwendeter Farbstoffe. *Ibid.*, CXVI., 348.

FISCHL, V., and SINGER, E., 1935, Die Chemotherapie der Rattentrypanose. *Ibid.*, CXVI., 652.

FRANKE and ROEHL, 1907, quoted by Ehrlich, P., Chemotherapeutische Trypanosomen-Studien. *Berl. klin. Wschr.*, XLIV., 233.

GONDER, R., 1911, Untersuchungen über arzneifeste Mikroorganismen. I. *Trypanosoma lewisi*. *Zbl. Bakt.* Abt. I. Orig., LXI., 102.

GONDER, R., 1912, Experimentelle Studien mit Trypanosomen und Spironemen (Spirochäten). *Z. ImmunForsch.*, XV., 257.

HASSKÓ, A., 1932, Beiträge zur Kenntnis des chemotherapeutischen Interferenzphänomens. *Z. ges. exp. Med.*, LXXXIII., 792.

HAWKING, F., 1937, Studies on chemotherapeutic action. I. The absorption of arsenical compounds and tartar emetic by normal and resistant trypanosomes and its relation to drug resistance. *J. Pharmacol.*, LIX., 123.

JADIN, J., 1932, La photo-sensibilité de quelques parasites. *C.R. Soc. Biol., Paris*, CX., 124.

JANSSENS, P. G., 1936, Contribution expérimentale à l'étude de l'influence du chauffage au bain-marie et des ondes courtes, seules ou en association chimiothérapique, sur une souche arsénorésistante de *Tr. gambiense*. *Rev. belge Sci. méd.*, VIII., 400.

LAUNOY, L., 1937, Chimio-résistance de *T. annamense* à la suite d'une seule injection massive de tryparsamide à un chat infecté. *Bull. Soc. Path. exot.*, XXX., 234.

LESTER, H. M. O., 1932, The influence of cyclical transmission by *Glossina tachinoides* on a strain of *Trypanosoma brucei* made resistant to human serum. *Ann. trop. Med. Parasit.*, XXVI., 525.

LEUPOLD, F., 1925, Die Bedeutung der Blepharoplasten als Angriffspunkt chemotherapeutischer Substanzen. *Z. Hyg. InfektKr.*, CIV., 641.

LOURIE, E. M., and YORKE, W., 1938, Studies in chemotherapy. XX. The preparation of strains of trypanosomes resistant to synthelin and undecane diamidine and an analysis of their characters. *Ann. trop. Med. Parasit.*, XXXII., 201.

MESNIL, F., and BRIMONT, E., 1908, Sur les propriétés des races de trypanosomes résistantes aux médicaments. *Ann. Inst. Pasteur*, XXII., 856.

MURGATROYD, F., and YORKE, W., 1937, Studies in chemotherapy. XIV. The stability of drug-resistance in trypanosomes. *Ann. trop. Med. Parasit.*, XXXI., 165.

MURGATROYD, F., YORKE, W., and CORSON, J. F., 1937, Studies in chemotherapy. XIII. The changes observed in *T. brucei* during five years' maintenance in the laboratory. *Ibid.*, XXXI., 145.

NAITO, T., and OKA, S., 1936, Untersuchungen ueber die Festigkeit von Trypanosomen gegen Arsenpräparate. *Zbl. Bakt.*, Abt. I. Orig., CXXXVII., 401.

PEDLOW, J. T., and REINER, L., 1935, Comparison of the binding of chemotherapeutic agents by normal and resistant trypanosomes. *J. Pharmacol.*, LV., 179.

REICHENOW, E., and REGENDANZ., P., 1927, Ueber die Flohpassage normaler und mit Arsenophenylglycin vorbehandelter Rattentrypanosomen. *Abh. Auslandsk.*, XXVI., 446.

REINER, L., LEONARD, C. S., and CHAO, S. S., 1932[1], Studies on the mechanism of chemotherapeutic action: on binding of arsenicals by trypansomes *in vitro*. *Arch. int. Pharmacodyn.*, XLIII., 186.

REINER, L., LEONARD, C. S., and CHAO, S. S., 1932[2], Studies on the mechanism of chemotherapeutic action: on binding of arsenicals by arsenic-resistant trypanosomes *in vitro*. *Arch. int. Pharmacodyn.*, XLIII., 199.

ROBERTSON, M., 1929, The action of acriflavine upon *Bodo caudatus*. A study of heritable modification in a non-conjugating protozoan and its relation to certain aspects of chemotherapy in trypanosomiasis. *Parasitology*, XXI., 375.

ROEHL and GULBRANSEN, 1909, quoted by Ehrlich, P. *Arch. Schiffs- u. Tropenhyg.*, XIII, Beiheft 6, 91.

SCHERN, K., and ARTAGAVEYTIA-ALLENDE, R., 1936, Zur glykopriven Therapie und Prophylaxie mit sowohl toxisch als auch atoxisch wirkenden Substanzen bei der experimentellen Trypanosomen- und Treponemen-Infektion. *Z. ImmunForsch.*, LXXXIX., 21.

SCHILLING, C., SCHRECK, H., NEUMANN, H., and KUNERT, H., 1936, Versuche zur Schutzimpfung gegen Tsetse-Krankheit. *Ibid.*, LXXXIX., 279.

SCHLOSSBERGER, H., and SCHÜFFNER, R., 1934, Festigungsversuche an Trypanosomen mit Arsenpyridinderivaten. *Angew. Chem.*, XLVII., 768.

SINGER, E., and FISCHL, V., 1934, Der Nachweis von Arsenikalien in Spirochäten und Trypanosomen. *Z. Hyg. InfektKr.*, CXVI., 36.

SINGER, E., and FISCHL, V., 1935, Arzneifestigkeit und Chemikaleingewöhnung der Trypanosomen. *Ibid.*, CXVI., 683.

VON JANCSÓ, N., 1931, Photobiologische Studien in der Chemotherapie. I. Lichtempfindliche Trypanosomen in der trypaflavinbehandelten Tieren. *Zbl. Bakt.* Abt. I. Orig., CXXII., 388.

VON JANCSÓ, N., 1932, Photobiologische Studien in der Chemotherapie. III. Therapeutische und photodynamische Festigung von Naganatrypanosomen durch systematische Behandlung mit Trypaflavin. *Zbl. Bakt.*, Abt. I. Orig., CXXIII., 129.

von Jancsó, N., and von Jancsó, H., 1935[1], The rôle of the natural defence forces in the evolution of the drug-resistance of trypanosomes. II. The rapid production of germanin-fast *T. brucei* strains in animals with paralysed defence. *Ann. trop. Med. Parasit.*, **XXIX.**, 95.

von Jancsó, N., and von Jancsó, H., 1935[2], Chemotherapeutische Schnellfestigung von Trypanosomen durch Ausschaltung der natürlichen Abwehrkräfte. *Z. ImmunForsch.*, **LXXXV.**, 81.

Werbitzki, F. W., 1910, Ueber blepharoplastlose Trypanosomen. *Zbl. Bakt.* Abt. I. Orig., **LIII.**, 303.

Yorke, W., 1932, Drug resistance with special reference to trypanosomiasis. *Brit. med. J.*, *ii*, 668.

Yorke, W., 1934, Die Probleme der Arzneifestigkeit *Arch. Schiffs- u. Tropenhyg.*, **XXXVIII.**, 55.

Yorke, W. Adams, A. R. D., and Murgatroyd, F., 1929, Studies in chemotherapy. I. A method for maintaining pathogenic trypanosomes alive *in vitro* at 37° C. for twenty-four hours. *Ann. trop. Med. Parasit.*, **XXIII.**, 501.

Yorke, W., and Hawking, F., 1932, Studies in chemotherapy. VII. Is the resistance of a drug-fast trypanosome modified by transference to a different species of vertebrate host ? *Ibid.*, **XXVI.**, 215.

Yorke, W., and Murgatroyd, F., 1930, Studies in chemotherapy. III. The action *in vitro* of certain arsenical and antimonial compounds on *T. rhodesiense* and on atoxyl- and acriflavine-resistant strains of this parasite. *Ibid.*, **XXIV** , 449.

Yorke, W., Murgatroyd, F., and Hawking, F., 1931, Studies in chemotherapy. V. Preliminary contribution on the nature of drug resistance. *Ibid.*, **XXV.**, 351.

Yorke, W., Murgatroyd, F., and Hawking, F., 1932[1], Studies in chemotherapy. VIII. Comparison of strains of *T. rhodesiense* made resistant to various arsenicals and antimonials, to Bayer 205 and to acriflavine respectively. *Ibid.*, **XXVI.**, 577.

Yorke, W., Murgatroyd, F. and Hawking, F. 1932[2], Studies in chemotherapy. IX. Sodium thiosulphate and arsenic resistance. *Ibid.*, **XXVI.**, 587.

Yorke, W., Murgatroyd, F., and Hawking, F., 1933 [1], Effect of passage through *Glossina* on the resistance of a tryparsamide-fast trypanosome. *Brit. med. J.*, *i*, 176.

Yorke, W., Murgatroyd, F., and Hawking, F., 1933 [2], Studies in chemotherapy. X. Further observations on the transmissibility of tryparsamide-resistance by *Glossina*. *Ann. trop. Med. Parasit.*, **XXVII.**, 157.

CHEMOTHERAPEUTIC INTERFERENCE

In view of the fact that drug resistance is associated with a decreased power of absorption of the drug by the resistant trypanosomes the phenomenon brought to light by Browning and Gulbransen (1922), and termed "therapeutic interference," takes on a new interest. It was found that when mice are fed with parafuchsin and are subsequently inoculated with a strain of trypanosome resistant to parafuchsin the therapeutic action of trypaflavine is greatly reduced. These observations were confirmed by Schnitzer (1926), who showed that to demonstrate interference it is not necessary to employ trypanosomes which are resistant to parafuchsin. Schnitzer and Rosenberg (1926 and 1927) and Schnitzer and Silberstein (1926) in addition found that parafuchsin injected along with certain compounds, or preferably a few hours before, interfered with the chemotherapeutic action of trypaflavine, arsacetin, arsphenamine and tartar emetic, while pyoktanin (a mixture of penta- and hexamethyltriaminotriphenylmethane hydrochloride) interfered with the action of trypaflavine, arsacetin, arsphenamine and tartar emetic but not with tryparosan (Schnitzer and Silberstein, 1927). Browning *et al.* (1931) showed that in the production of certain quaternary salts of styrylquinolines the methods of preparation may lead to the formation of isomerides, which are not only less trypanocidal but interfere with the chemotherapeutic action of the actively trypanocidal compound. Fischl and Fischl (1934) observed an interference effect on the part of ascorbic acid with tartar emetic, arsenophenylglycine, *m*-amino-*p*-oxyphenylarsenoxide and trypaflavine. Hasskó (1935) found that preliminary injections of methyl violet, ethyl violet or pyoktanin greatly lessened the capacity of the trypanosomes to absorb acriflavine, while a preliminary injection of trypan red or trypan blue almost entirely prevented the absorption of acriflavine by the trypanosomes. Von Jancsó (1931) showed that after a preliminary injection of parafuchsin, trypaflavine did not render the trypanosomes photo-sensitive to the same degree as untreated trypanosomes.

These experiments demonstrate that the phenomenon of chemotherapeutic interference is associated not with any change in the tissues of the host, but, as in the case of drug resistance, with decreased absorption by the parasites. Wright and Hirschfelder (1930) have in fact shown that the chemotherapeutic interference associated with trypanosomes can be duplicated *in vitro* in cultures of yeasts. Acriflavine interferes with the carbon dioxide production of yeasts, but when the yeast cells are stained by methyl violet or brilliant green in solutions too weak to affect carbon dioxide production, they become less sensitive to the action of acriflavine, while yeasts stained with dilute solutions of acriflavine are less sensitive to methyl violet and brilliant green. The reaction appears to be due to adsorption of the first dye on the surface of the cell interfering with adsorption of the second dye.

Chemotherapeutic interference is, however, similar in certain ways to the phenomenon described by Thung (1931) and Salaman (1933), who found that in plants the injection of a feebly pathogenic strain of a virus protein protected the plants against the subsequent injection of a highly pathogenic strain of the same virus protein. Findlay and MacCallum (1937) and Dalldorf, Douglass and Robinson (1938) have shown that animal viruses of low pathogenicity may on occasions protect against other animal viruses of high pathogenicity. It is obvious that though in certain cases closely allied substances may exhibit the interference phenomenon, in others there is no obvious chemical relationship between the interfering substance and the compounds with whose action it interferes.

In order to explain chemotherapeutic interference von Jancsó and von Jancsó (1936) have advanced the theory that the combinations of drugs which produce interference are only those which form thermodynamically reversible redox systems, the redox potential of which belongs to a definite potential range. The interference action is accordingly a characteristic attribute of electro-active bodies which readily store up and give off electrons. Thus in a series of twenty quinonoid redox dyes with graduated normal potentials, the activity varies gradually with the value of the normal potential. The weakly positive and negative dyes between the potential points of the redox scale + 0·115 and — 0·060 volt (*p*H 7), namely toluylene blue, thionin, cresyl blue, gallocyanin, toluidin blue, azur, methylene blue, pyocyanin, janus green, æthylcapri blue, all exhibit a pronounced interference action. A distinct optimum is reached at + 0·011 volt : toluidin blue and especially azur I inhibit the action of even large doses of arsenoxide and fuadin. Eight dyes, the normal potential of which is below — 0·060, were inactive. Ascorbic acid exerts a strong interference action against fuadin. This is explained by the fact that ascorbic acid forms a reversible redox system. The interference of such dyes as toluidin blue, cresyl blue, janus green and azur I on the trypanocidal action of arsenoxide was also confirmed by *in vitro* experiments, the action, for instance, of a 1 : 30,000 solution of arsenoxide, which normally killed trypanosomes in one or two minutes, was so far inhibited by the dyes that the parasites were still actively motile after fifteen or twenty minutes. Von Jancsó and von Jancsó believe that the dyes which interfere with the action of tervalent arsenicals and antimonials act as accessory respiratory catalysts and prevent damage to the respiratory system of the trypanosomes. This suggestion receives support from the work of Scheff and Hasskó (1936), who found that trypanosomes which had been protected by chemotherapeutic interference showed no decrease in oxygen or sugar consumption as compared with trypanosomes treated only by the toxic drug.

References

BROWNING, C. H., COHEN, J. B., COOPER, K. E., GULBRANSEN, R., 1931, Therapeutic interference caused by isomerides of trypanocidal styryl quinoline derivatives. *Proc. roy. Soc.*, B, CIX., 51.

BROWNING, C. H., and GULBRANSEN, R., 1922, An interference phenomenon in the action of chemotherapeutic substances in experimental trypanosome infections. *J. Path. Bact.*, XXV., 395.

DALLDORF, G., DOUGLASS, M., and ROBINSON, H. E., 1938, The sparing effect of canine distemper on poliomyelitis in *Macaca mulatta*. *J. exp. Med.*, LXVII., 333.

FINDLAY, G. M., and MACCALLUM, F. O., 1937, An interference phenomenon in relation to yellow fever and other viruses. *J. Path. Bact.*, XLIV., 405.

FISCHL, V., and FISCHL, L., 1934, Arzneifestigkeit, Avidität, Interferenz. *Z. ImmunForsch.*, LXXXIII., 324.

HASSKÓ, A., 1935, Untersuchungen ueber den Wirkungsantagonismus chemotherapeutischer Mittel II. *Z. Hyg. InfektKr.*, CXVI., 669.

SALAMAN, R. N., 1933, Protective inoculation against a plant virus. *Nature*, CXXXI., 468.

SCHEFF, G., and HASSKÓ, A., 1936, Einfluss einiger Chemotherapeutika auf den Stoffwechsel der Trypanosomen, mit Rücksicht auf das Interferenzphänomen. *Zbl. Bakt.* Abt. I. Orig., CXXXVI., 420.

SCHNITZER, R., 1926, Untersuchungen zur Chemozeptorentheorie. I. Das Interferenzphänomen von Browning und Gulbransen. *Z. ImmunForsch.*, XLVII., 116.

SCHNITZER, R., and ROSENBERG, E., 1927, Untersuchungen zur Chemozeptortheorie. IV. Vergleich des Interferenzphänomens au parafuchsinfesten und normalen Trypanosomen. *Ibid.*, XLIX., 393.

SCHNITZER, R., and ROSENBERG, E., 1926, Untersuchungen zur Chemozeptorentheorie. II. Die Hemmungswirkung des Parafuchsins gegenüber trypanoziden Arzenikalien. *Ibid.*, XLVIII., 23.

SCHNITZER, R., and SILBERSTEIN, W., 1926, Untersuchungen zur Chemozeptorentheorie. III. Die Hemmungswirkung des Parafuchsins gegenüber Brechweinstein. *Ibid.*, XLIX., 387.

SCHNITZER, R., and SILBERSTEIN, W., 1927, Untersuchungen zur Chemozeptorentheorie. VI. Mitteilung. Der "Sulfhydryl-Chemozeptor." Seine Beziehungen zum Interferenzphänomen. *Ibid.*, LIII., 439.

THUNG, T. H., 1931, Handeling 6. Ned.-Indisch Natuurwetensch. Congr. 22-26 Sept., Bandoeng (Java), p. 405.

VON JANCSÓ, N., 1931, Photobiologische Studien in der Chemotherapie. II. Photodynamisches und therapeutisches Interferenzphänomen bei der experimentellen Naganainfektion. *Zbl. Bakt.* Abt. I. Orig., CXXII., 393.

VON JANCSÓ, N., and VON JANCSÓ, H., 1936, Beziehungen zwischen chemotherapeutischer Wirkung, Oxydationskatalyse und Redoxpotential. *Z. ImmunForsch.*, LXXXVIII., 275.

WRIGHT, H. N., and HIRSCHFELDER, A. D., 1930, Studies on the colloid chemistry of antisepsis and chemotherapy. IV. The duplication *in vitro* of the interference phenomenon in combination chemotherapy. *J. Pharmacol.*, XXXIX., 39.

THE CHEMOPROPHYLAXIS OF SLEEPING SICKNESS

Chemoprophylaxis may be due to the fact that, though rapidly excreted or destroyed, the chemical compound may lead to a long persisting elevation of the defensive powers of the tissues. In the case of trypanosomiasis there is no evidence in support of this mode of action. The compound may be excreted or destroyed so slowly that it continues to circulate and exert a trypanocidal activity for a long period, or finally it may form a relatively insoluble local deposit in the tissues from which small amounts continually pass into the body fluids to act on the parasites.

In order to be of value as a chemoprophylactic against sleeping sickness for any length of time, a drug, it appears, must maintain a trypanocidal titre in the serum. Since the tervalent arsenicals and arsphenamines produce a high trypanocidal titre in the serum for only a comparatively short period they should, on theoretical grounds, be of little value in prophylaxis, and this, in fact, has been shown to be true experimentally. Quinquevalent arsenicals, on the other hand, are present in the serum in a trypanocidal titre for a much longer period and, as was shown by Levaditi, Nicolau and Galloway (1926), tryparsamide exerts an efficient prophylactic action against nagana infections when given by mouth to rabbits. The effect of tryparsamide in preventing sleeping sickness in man has been tested in the field by Fourche and Haveaux (1931), who gave 2 gm. of tryparsamide intravenously. The period of protection lasted for approximately six months and the number of fresh infections among those prophylactically treated was twelve times less than among the untreated controls. Even so, the results were not as successful as with germanin. The prophylactic action of germanin is due to the fact that concentrations in the blood of 10 mgm. per 100 c.c. and not infrequently as low as 1 mgm. per 100 c.c. are sufficient to inhibit the power of trypanosomes to infect the mouse. As Dangerfield, Gaunt and Wormall (1938) have shown, concentrations of 1 mgm. of germanin per 100 c.c. can usually be detected in the blood five to six months after intravenous injection of a rabbit with 100 mgm. per kilogram of body weight. The amount of germanin in the blood is usually greater when a number of small doses of germanin are given over a period of a few weeks than when the same amount of the drug is given in a single injection.

In laboratory animals the prophylactic action of germanin or moranyl varies somewhat with the species of animal employed. In the mouse Launoy, Nicolle and Prieur (1929 [1 & 2]) have shown that the length of time for which a mouse is protected by moranyl varies directly with the amount of the drug injected. The same appears to be true in the cat.

THE RELATIONSHIP BETWEEN THE DOSE OF MORANYL (FOURNEAU 309) AND THE LENGTH OF TIME FOR WHICH THE MOUSE IS PROTECTED AGAINST TRYPANOSOME INFECTION

	T. brucei		*T. evansi*		*T. equiperdum*	
Dose in gm. (intravenous) for a mouse of 20 gm. body weight	Minimal prevention in days	Maximal prevention in days	Minimal prevention in days	Maximal prevention in days	Minimal prevention in days	Maximal prevention in days
0·0005	0	3	2	3	1	4
0·00010	3	5	5	3	8	10
0·00020	3	10	15	26	15	25
0·00030	10	16	15	31	20	25
0·00040	10	25	33	40	33	40
0·00050	25	40	35	40	33	40
0·00060	31	41	35	41	40	40

In rats, Corson (1934) found that a dose of 0·015 gm. per kilogram of body weight did not protect them for twenty-one days against the bites of tsetse flies infected with a recent strain of *T. rhodesiense*, while a dose of 0·03 gm. per kilogram of body weight failed to protect for forty days.

In monkeys, Kleine and Fischer (1922) showed that the injection of 0·15 gm. protected in one instance for one month and in another for two months against infection from the bites of wild flies. Duke (1934) gave injections of from 0·158 gm. to 0·023 gm. per kilogram of body weight to three monkeys: these animals became sensitive to the bites of tsetse flies infected with *T. rhodesiense* after sixty-seven, sixty-nine and seventy-four days respectively.

In domestic animals germanin has been employed prophylactically in a variety of trypanosome infections. For prophylaxis against dourine in horses, Pfeiler (1920) recommended 6 gm. during the covering season in two doses of 3 gm. administered at an interval of eight days. Infection with *T. venezuelense* is inhibited, according to Tejera (1924), by a dose of 2 gm. repeated every six months. The results obtained by Kleine (1923) and Berg (1925) in cattle were disappointing, for even 10 gm. for a bullock of 5 cwt. in repeated doses failed to prevent infection. Against surra, Baermann (1922) found that protection was obtained for about thirty to forty days by injecting 2 to 6 gm. of germanin. Edwards (1926) also obtained complete protection against surra for a period of twenty-two days by an intravenous injection of 7·5 gm. per 1,000 lbs. of body weight.

In man, Duke (1934) showed conclusively that a single dose of 1 gm. of germanin given intravenously will protect a man against infection with *T. rhodesiense* transmitted by the bites of tsetse flies, a second dose three weeks later enhances the protective action. The protection afforded appears to vary somewhat with different strains of *T. rhodesiense*, possibly because of the differing power which these strains possess of propagating in man. The protection against *T. rhodesiense* is greater than against *T. gambiense*. Duke, therefore, recommends that an injection of from 1·0 to 1·5 gm. of germanin should be repeated every three months. This agrees closely with the earlier conclusion of Mayer (1928) that Europeans should receive intravenous injections of 2 gm. every three months and native Africans similar injections of 1 gm.

The prophylactic use of germanin and orsanine in man has now been studied on a large scale in many areas in Africa, chiefly by French and Belgian investigators. Earlier results are summarised by Mayer (1928). Arnaud (1929) working in a highly infected area studied the effect of injections of moranyl on 450 persons. Thirteen months after the prophylactic injections the number of fresh infections was noted. A close correlation was found between the dose administered and the number of new cases.

THE EFFECT OF DOSAGE ON THE PROPHYLACTIC ACTION OF MORANYL
(Arnaud, 1929)

Dose in gm. per kilogram of body weight	No. of persons injected	No. of new cases	Percentage of new cases
0·02 . . .	25	0	0
0·02 to 0·015 . .	57	4	7
0·015 to 0·010 .	247	41	17
less than 0·010 .	121	39	32

Fourche and Haveaux (1931) found that of a population given 2 gm. of germanin in two doses, only 0·09 per cent. had become infected thirteen months later, while of controls not prophylactically treated, 4·1 per cent. had contracted sleeping sickness.

The evidence is thus strongly in favour of the view that germanin acts as a true chemotherapeutic prophylactic against trypanosome infections both in man and in animals; this action is due to the fact that germanin continues to be present in the blood-stream. Whether or not this is due to the continued liberation of germanin from tissue deposits still seems uncertain.

In the case of certain benzoylaminostyrylquinoline compounds which have been found by Browning and Gulbransen (1934) to exert a prolonged prophylactic action against trypanosomes in mice, the protective power is due to the fact that when a watery solution is injected subcutaneously a local deposit forms, accompanied by considerable reaction on the part of the connective tissue elements. The compounds are fixed intracellularly and after an injection of 1 c.c. of a 1 in 200 or 1 in 250 solution of 2(*p*-acetylaminostyryl)-6(*p*-aminobenzoylaminoquinoline methoacetate) the drug still persists at the site of inoculation and still protects the animal from infection for almost a year. Though the amount of the drug present in the circulation at any one time is comparatively small, it is sufficient to prevent infection. While striking prophylactic action is associated with the presence of *p*-aminobenzoyl, *p*-dimethylaminobenzoyl and *p*-acetylaminobenzoyl groups substituted in the 6-NH_2 group of the quinoline nucleus, the analogous trypanocidal substances in which the 6-position is occupied by $—N(CH_3)_2$ or $—NH.CO.CH_3$ exert only a brief prophylactic action. This can be correlated with the fact that after subcutaneous injection they are rapidly absorbed and rapidly excreted.

References

ARNAUD, R., 1929, Au sujet de moranyl. Deux opérations de moranylisation dans le Moyen-Congo. *Bull. Soc. Path. exot.*, XXII., 872.

BAERMANN, G., 1922, Die Behandlung der Surra mit "Bayer 205." *Beihefte Arch. Schiffs-u. Trop. Hyg.*, XXVI., 4.

BERG, H., 1925, Die Eignung von "Bayer 205" zur Bekämpfung der afrikanischen Haustiertrypanosomen. *Dtsch. tierärztl. Wschr.*, XXXIII., 561.

BROWNING, C. H., and GULBRANSEN, R., 1934, Prophylaxis of experimental trypanosome infections by chemotherapeutic agents. *J. Path. Bact.*, XXXIX., 75.

CORSON, J. F., 1934, The action of "Bayer 205" on *Trypanosoma rhodesiense* in white rats infected by tsetse flies. *Ann. trop. Med. Parasit.*, XXVIII., 535.

DANGERFIELD, W. G., GAUNT, W. E., and WORMALL, A., 1938, Studies on Bayer 205 (Germanin) and Antrypol. I. The determination of small amounts of Bayer 205 (and Antrypol). II. The persistence of Bayer 205 in the blood-stream after injection into animals. *Biochem. J.*, XXXII., 59.

DUKE, H. L., 1934, On the protective action of "Bayer 205" against the trypanosomes of man. *Lancet i*, 1336.

EDWARDS, J. T., 1926, The chemotherapy of surra (*Trypanosoma evansi* infections) of horses and cattle in India. *Trans. R. Soc. trop. Med. Hyg.*, XX., 10.

FOURCHE, J. A., and HAVEAUX, G., 1931, Germanine et tryponarsyl appliqués préventivement contre la trypanosomiase. *Bull. Soc. Path. exot.*, XXIV., 557.

KLEINE, F. K., 1923, On a recent expedition to Africa to investigate the action of Bayer 205 in trypanosomiasis. *Trans. R. Soc. Med. Hyg.*, XVII., 445.

KLEINE, F. K., and FISCHER, W., 1922, Bericht über die Prüfung von "Bayer 205" in Afrika. *Dtsch. med. Wschr.*, XLVIII., 1693.

LAUNOY, L., NICOLLE, P., and PRIEUR, M., 1929[1], Détermination des doses liminaires préventives du composé 205-Bayer-309-Fourneau dans quelques trypanosomiases expérimentales. *C.R. Soc. Biol., Paris*, CI., 650.

LAUNOY, L., NICOLLE, P., and PRIEUR, M., 1929[2], Nouveaux documents sur l'action préventive du 309 dans le nagana expérimental de la souris. *Ibid.*, CII., 663.

LEVADITI, C., NICOLAU, S., and GALLOWAY, I., 1926, Essais de prophylaxie des trypanosomiases par des dérivés phényl-arseniques administrés *per os*. *Bull. Soc. Path. exot.*, XIX., 737.

MAYER, M., 1928, Die Behandlung und Prophylaxie der afrikanischen Schlafkrankheit mit Germanin (" Bayer 205 "). Kritische Besprechung der bisherigen Ergebnisse und Vorschläge. *Arch. Schiffs- u. Tropenhyg.*, XXXII., 528.

PFEILER, W., 1920, Prophylaxie bei Beschälseuche. *Mitt. Tierseuchenst., Jena*, II., 13.

TEJERA, E., 1924, Investigaciones hechas con el 205 en el tratamiento de la tripanosomiasis que ocasiona le " derrengadera " de los equinos de Venezuela. *Gac. méd, Caracas,* XXXI., 177.

THE CHEMOTHERAPEUTIC ACTION OF DRUGS IN TRYPANOSOMIASIS

Despite the intensive study of trypanosome infections and their reaction to drugs, a number of problems still require further study before any true picture is obtainable of the means by which trypanosomes are destroyed by drugs. Thus, in view of the fact that the distribution of any species of pathogenic trypanosome within the tissues of its host at any stage of infection is unknown, it is usually impossible to state the situation where the destruction of trypanosomes by drug action takes place. Then also it is still uncertain whether sterilisation is ever due to drug action alone, or to the combined action of drugs and immune bodies.

In considering the means by which trypanosomes are destroyed by drugs in the body a number of phenomena require consideration:

(1) The development of trypanocidal activity *in vitro* and *in vivo*.

(2) The rate of absorption and excretion of trypanocidal substances by the tissues.

(3) The action of trypanocidal drugs on trypanosomes.

(4) The specific relationship between arsenical compounds and certain trypanosome infections.

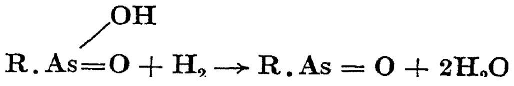
(5) Synergic action.

(6) Spontaneous changes in the reaction of strains of trypanosomes to drugs.

(7) The rôle of the reticulo-endothelial system.

(1) The Development of Trypanocidal Activity *in vitro* and *in vivo*

Since the arsonic acids are relatively inactive in the destruction of trypanosomes *in vitro* but active *in vivo*, while the arsenoxides are active *in vitro*, it was thought by Ehrlich (1909) that the tissues must have the power of reducing the quinquevalent arsonic acids to the tervalent arsenoxide which then destroys the trypanosomes :—



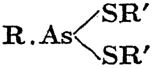
$$\mathrm{R.\overset{\diagup OH}{As}{=}O + H_2 \rightarrow R.As = O + 2H_2O}$$

Arsenical compounds were thus divided into the quinquevalent arsonic acids on the one hand and the tervalent compounds, arsenoxides and arseno-compounds on the other.

This theory, which is now generally accepted, was extended by Voegtlin and Smith (1920 [1 & 2]) and Voegtlin, Dyer and Leonard (1923), who showed that *in vivo* the time required for quinquevalent arsenic and antimony derivatives to exert trypanocidal activity is longer than for tervalent compounds. This longer latent period, it was assumed, must be due to the gradual reduction of a sufficient amount of the drug to the tervalent form. Since also arsenic in the arsenoxide form acts much more rapidly than arsenic in the form of an arsphenamine derivative it is suggested that these latter compounds are oxidised in the body to the oxide :—

$$\mathrm{R.As = As.R + O_2 \qquad\qquad = O}$$

This conception is strengthened by the fact that the toxicity of a slightly alkaline aqueous solution of arsphenamine or neoarsphenamine is increased when kept in contact with air, probably owing to oxidation to *meta*-amino-*para*-hydroxyphenylarsenoxide, since, on treating arsphenamine in alkaline solution with hydrogen peroxide, Ehrlich and Bertheim (1912) produced *meta*-amino-*para*-hydroxyphenylarsonic acid. In addition, an aqueous solution of the sodium salt of arsphenamine which has been incubated at 37° C. for three hours exerts a much greater trypanocidal activity than freshly prepared solutions and has no latent period in its trypanocidal action. Since reduction of the quinquevalent arsenicals to the arsenious oxide stage would require a longer time than oxidation of the arsphenamines to the same stage, a longer latent period for trypanocidal action would be necessary for the arsonic acids than for the arsphenamines. This has been found to be the case by Voegtlin and Smith (1920 [2]), the latent period for the arsphenamines being from one to three hours, for the quinquevalent arsonic acids from twelve to eighteen hours.

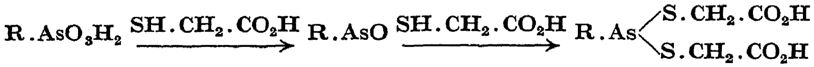
The difference in action of quinque- and tervalent arsenicals has been reinvestigated by Yorke and Murgatroyd (1930), employing the method elaborated by Yorke, Adams and Murgatroyd (1929) for maintaining pathogenic trypanosomes alive and active *in vitro* at 37° C. for at least twenty-four hours. It was found that whereas the quinquevalent compounds had very little trypanocidal action *in vitro*, all compounds containing tervalent arsenic, whether arsenoxides of the type $\mathrm{R.As = O}$, thioarsinites  $\mathrm{R.As}\left\langle\begin{matrix}\mathrm{SR'}\\ \mathrm{SR'}\end{matrix}\right.$ or arseno-compounds $\mathrm{R.As = As.R}$ were lethal to trypanosomes in high dilution. Like Ehrlich, Yorke and his colleagues thus divide arsenical compounds into two groups, the weakly toxic arsonic acids and the intensely toxic tervalent compounds, the therapeutic effect of which, however, they ascribe to the direct trypanocidal action of the unchanged drugs. So far as quinquevalent arsenicals are concerned there is general agreement that their toxicity *in vitro* is small not only to trypanosomes but to spermatozoa and to cells in tissue culture (*cf.* Levaditi and Constantinesco, 1932), whereas, as Yorke and Murgatroyd (1930) have shown, the minimal lethal concentration of quinquevalent arsenicals *in vitro* for *T. rhodesiense* is 1 in 1,600, while reduction to the tervalent form increases the minimal lethal concentration to 1 in 200,000,000.

Since quinquevalent arsenic is thus only weakly trypanocidal *in vitro* but becomes active *in vivo*, the question naturally arises how this activation is brought about. The work of Levaditi and Yamanouchi (1908) and Levaditi (1909 [1&2]) showed that when atoxyl was incubated at 37° C. with an emulsion of liver, kidney, brain or muscle, trypanocidal properties arose in the mixture, due to the formation of a hypothetical substance "trypanotoxyl." Yamanouchi (1910) concluded that the trypanocidal substance was produced by the red blood corpuscles because the liver and other organs freed from blood no longer possess the power of activating atoxyl. Red cells acted more powerfully in the presence of an excess of carbon dioxide than under normal conditions, while in the presence of oxygen they failed altogether to activate atoxyl : pure recrystallised hæmoglobin was without action. The active substance was soluble in alcohol, thermostable and free from protein. Terry (1912) found that both liver and blood, when incubated with atoxyl, transformed the drug into a highly toxic substance. The transforming agent in liver had, however, characteristics, which, in some respects, differed from those of the active agent in blood. Terry (1915) also believed that the transforming agent in blood is thermolabile, while the toxic substance, into which atoxyl is transformed, is thermostable. Levaditi, Anderson and Manin (1928) suggested that the constituent in the tissues which converts atoxyl into a trypanocidal substance is reduced glutathione, a view also taken by Singer and Fischl (1934). However, the relationship between the glutathione content of the organs and their capacity for transforming atoxyl into trypanotoxyl is neither absolute nor constant. It is therefore argued that there must be a second factor operating in addition to glutathione, since when atoxyl and tissues are incubated together, some glutathione always remains in the tissues and even after extracting the tissues with trichloracetic acid, which removes glutathione, the tissues retain the power of forming trypanotoxyl. Furthermore, blood which is poor in glutathione activates atoxyl.

The relationship of the blood cells to activation of quinquevalent arsenicals has also been studied by Lourie, Murgatroyd and Yorke (1935). It was found that both reduced tryparsamide and tryparsamide diffuse into red cells and that that part of the tryparsamide which enters into the red cells is changed into a more actively trypanocidal substance. Although a solution of laked red blood corpuscles greatly increases the trypanocidal power of tryparsamide, the agent activating tryparsamide is neither reduced hæmoglobin, oxyhæmoglobin nor carboxyhæmoglobin. The activating substance is relatively thermostable, resisting almost completely a temperature of 65° C. for thirty minutes and is not completely destroyed by heating at 75° C. for thirty minutes. The activating power of red blood corpuscle solutions kept at 0° C. is, however, gradually lost so that within two months or less such solutions become practically inert.

While the evidence is thus considerable that the quinquevalent arsenicals are reduced in the body to arsenoxides there is still some uncertainty as to whether the thioarsinites and arsphenamines exert their curative action in the unchanged condition, or after oxidation to the active arsenoxide R.AsO.

Friedberger (1908) foreshadowed the significance of the thioarsinites as chemotherapeutic agents when he found that *p*-aminophenylarsonic acid mixed with thioglycollic acid became more toxic to mice as well as trypanocidally active *in vitro*. Bertheim (quoted by Roehl, 1909) showed by analogy with arsenious acid that this was due to the production of arsenoxide by reduction, with subsequent formation of thioarsinite, as shown in the following :—



$$R.AsO_3H_2 \xrightarrow{SH.CH_2.CO_2H} R.AsO \xrightarrow{SH.CH_2.CO_2H} R.As\begin{cases} S.CH_2.CO_2H \\ S.CH_2.CO_2H \end{cases}$$

Much later Voegtlin, Dyer and Leonard (1923) prepared di(carboxymethyl)3-amino-4-hydroxyphenyl thioarsinite by heating together 3-amino-4-hydroxyphenylarsenoxide and thiolactic acid. This compound showed a definite delay in trypanocidal action as compared with the parent arsenoxide. More recently Strangeways (1937) has studied the action of a number of thioarsinites prepared by condensation of aromatic arsenoxides and sulphydryl compounds such as glutathione and cysteine (*cf.* Cohen, King and Strangeways, 1931 [1 & 2], 1932). All these compounds were less toxic than their arsenoxide content would suggest and in many cases a substance with a high therapeutic index was produced by condensation with a sulphydryl compound of an oxide with a low therapeutic index. Thioarsinites have a toxicity for trypanosomes *in vitro* in high dilution. This toxicity is due to the hydrolysis of these compounds in aqueous solution with liberation of the highly trypanocidal arsenoxide, as is shown by (1) the identity in the lethal activity of equimolecular concentrations of thioarsinite and its parent oxide, (2) the inhibition of the lethal action both of an arsenoxide and a thioarsinite in strong solution by the addition of 10 molecules excess of glutathione and the failure to obtain protection in higher dilutions owing to more extensive hydrolysis. The inhibitory action of an excess of glutathione on the therapeutic action of the thioarsinites, due to the inhibition of the formation of arsenoxide, is thus similar to the inhibitory action of glutathione on the chemotherapeutic action of the 3-amino-4-hydroxyphenylarsenoxide. But though the directly trypanocidal action of the thioarsinites is due to arsenoxide the fact that thioarsinites are more effective in experimental trypanosomiasis than the corresponding arsenoxides is explained by Strangeways (1937) as follows. When thioarsinites, which are readily obtained in solution as neutral sodium salts, are introduced into the blood-stream in relatively high concentrations (1 in 1,000 to 1 in 100) there is very little hydrolysis to the arsenoxide and sulphydryl components, as shown by a negligible nitroprusside reaction. After intravenous injection and subsequent dilution there will be some hydrolysis with liberation of free arsenoxide. This partial hydrolysis is rapid, since the trypanosomes begin to disappear from the blood-stream within half an hour after injection: but it is not immediately complete since the dose of a thioarsinite which can be tolerated is greater then would be expected from the calculated content of arsenoxide.

Neoarsphenamine has a trypanocidal action *in vitro* in low concentrations, a fact amply verified by the work of Yorke and Murgatroyd (1930), but the lethal concentrations are higher than those found for arsenoxides and thioarsinites. The addition of 10 molecules excess of glutathione to strong solutions of neoarsphenamine inhibits its lethal action, though a similar excess of glutathione is without effect on the toxicity of high dilutions. Moncorps and Bohnstedt (1934) found that concentrations of glutathione of 1 in 100 to 1 in 500 reduced the trypanocidal activity of a 1 in 700 dilution of neoarsphenamine both *in vivo* and *in vitro*. Cysteine has a similar protective action against neoarsphenamine to that of glutathione. This lends support to the suggestion that neoarsphenamine is absorbed by the trypanosomes and becomes oxidised, either within the body of the trypanosome or on its surface, to the highly trypanocidal arsenoxide. In support of this view are the observations of Gonder (1912), Castelli (1913) and Simić (1923) who found that both *in vitro* and *in vivo* trypanosomes which have been in contact with neoarsphenamine for from five to thirty minutes retain their motility but no longer infect mice. Papamarku (1927) found that if a mixture of trypanosomes and spirochætes are treated with varying concentrations of neoarsphenamine the trypanosomes are killed after two hours by a dilution of 1 in 10,000,000, while the spirochætes remain healthy for a number of hours in much higher concentrations. Spirochætes, however, were killed by dilutions of 1 in 500,000 after twenty-four hours and 1 in 20,000,000 after forty-eight hours. This difference in time of action of trypanosomes and spirochætes is explainable if trypanosomes are able to oxidise arseno compounds to the arsenoxide, while spirochætes are unable to accomplish this change. The production of arsenoxide by the cells of the host may also play a part in the therapeutic effect of neoarsphenamine. Simić (1923) has indeed suggested that this action of the host's cells explains the greater therapeutic efficiency and higher toxicity of neoarsphenamine when given by subcutaneous as compared with intravenous injection. Hasskó (1935) also believes that the cells of the host play a large part in activating neoarsphenamine since, when mice infected with trypanosomes were injected with neoarsphenamine, no neoarsphenamine could be demonstrated within the bodies of the trypanosomes.

So far as non-metallic compounds are concerned, trypanocidal activity appears to be due to the direct action of the compounds themselves. The trypanocidal activity of acriflavine, for instance, is unaffected by the presence of sulphydryl compounds.

In the case of germanin it was for long thought that some chemical change in its composition must occur in the body since its trypanocidal action *in vitro* appeared to be very slight, while *in vivo* it was highly active. Von Jancsó and von Jancsó (1934), however, were able to show that actually the trypanocidal action of germanin *in vitro* is intense, provided the drug is allowed to act on the trypanosomes for at least twenty-four hours. By a modification of the method employed by Yorke *et al.* (1929) it was found possible to keep trypanosomes alive and in good condition for from fifty to seventy hours; germanin is then found to be trypanocidal in a dilution of 1 in 80,000, while a concentration of

1 in 60,000 is sufficient to destroy all the parasites, but this action is only manifest after a latent period of twenty-four hours. Germanin, which, it is suggested, acts not directly on the trypanosomes but by interference with the nutrition of the parasites, thus differs strikingly from such drugs as the arsenoxides which have an immediate effect.

References

BERTHEIM, A., quoted by Roehl, W., 1909.

CASTELLI, 1913, quoted by Simić, T. V. (1923).

COHEN, A., KING, H., and STRANGEWAYS, W. I., 1931 [1], Arylthioarsinites. *J. Chem. Soc.*, 3043.

COHEN, A , KING, H., and STRANGEWAYS, W. I., 1931 [2], Aromatic arsonic acids containing amide groups. *Ibid.*, 3236.

COHEN, A., KING, H., and STRANGEWAYS, W. I., 1932, Arylthioarsinites from cysteine and glutathione. *Ibid.*, 1707.

EHRLICH, P., 1909, Über den jetzigen Stand der Chemotherapie. *Ber. dtsch. chem. Ges.*, XLII., 17.

EHRLICH, P., and BERTHEIM, A., 1912, Über das salzaure 3 : 3^1-Diamino-4 : 4^1-dioxy-arsenbenzol und seine nächsten Verwandten. *Ibid.*, XLV., 756.

FRIEDBERGER, E., 1908, Ueber die Behandlung der experimentellen Nagana mit Mischungen von Atoxyl und Thioglykolsäure. *Ber. klin. Wschr.*, XLV., 1714.

GONDER, R., 1912, Experimentelle Studien mit Trypanosomen und Spironemen (Spirochäten). *Z. Immun Forsch.*, XV., 257.

HASSKÓ, A., 1935, Experimentelle Beiträge zur Wirkungsweise chemotherapeutischer Mittel I. *Z. Hyg. InfektKr.*, CXVI., 660.

LEVADITI, C., 1909 [1], Mécanisme d'action des composés arsenicaux dans les trypanosomiases. *Bull. Soc. Path. exot.*, II., 45.

LEVADITI, C., 1909 [2], Le mécanisme d'action des dérivés arsenicaux dans les trypanosomiases. *Ann. Inst. Pasteur*, XXIII., 604.

LEVADITI, C., ANDERSON, T. E., and MANIN, Y., 1928, Le rôle du glutathion dans la production du trypanotoxyl et du bismoxyl. *Bull. Soc. Path. exot.*, XXI., 676.

LEVADITI, C., and CONSTANTINESCO, N., 1932, Etudes chimiothérapiques à l'aide des cultures cellulaires. Atoxyl et trypanotoxyl. *C.R. Soc. Biol., Paris*, CIX., 283.

LEVADITI, C., and YAMANOUCHI, T., 1908. Mécanisme d'action de l'atoxyl dans les trypanosomiasis. *Ibid.*, LXV., 23.

LOURIE, E. M., MURGATROYD, F., and YORKE, W., 1935, Studies in chemotherapy. XII. The diffusibility of the aromatic arsenicals into erythrocytes and the action of the latter on the pentavalent arsenicals. *Ann. trop. Med. Parasit.*, XXIX., 265.

MONCORPS, C., and BOHNSTEDT, R. M., 1934, Über den Einfluss des reduzierten Glutathion auf die kurative Neosalvarsan-Wirkung bei der naganakranken Maus. *Arch. Derm. Syph., Wien.*, CLXX., 26.

PAPAMARKU, P., 1927, Versuche über die Wirkung chemotherapeutischer Stoffe auf Spirochäten und Trypanosomen *in vitro*. *Z. Hyg. InfektKr.*, CVII., 407.

ROEHL, W., 1909, Ueber den Wirkungsmechanismus des Atoxyls. *Berl. klin. Wschr.*, XLVI., 494.

SIMIĆ, T. V., 1923, Untersuchungen über die Wirkungweise des Neosalvarsans. *Ibid.*, XCIX., 417.

SINGER, E., and FISCHL, V., 1934, Weitere Versuche ueber die Wirkung von Arzneimitteln *in vitro*. *Ibid.*, CXVI., 356.

STRANGEWAYS, W. I., 1937, The trypanocidal activity *in vitro* of aromatic thioarsinites and neoarsphenamine. *Ann. trop. Med. Parasit.*, XXXI., 387.

TERRY, B. T., 1912, The action of atoxyl. *Proc. Soc. exp. Biol., N.Y.*, IX., 41.

TERRY, B. T., 1915, Different amounts of transformed atoxyl produced by incubating 1 per cent. atoxyl in blood. *J. exp. Med.*, XXI., 258.

VOEGTLIN, C., DYER, H. A., and LEONARD, C. S., 1923, On the mechanism of the action of arsenic upon protoplasm. *Publ. Hlth. Rep., Wash.*, XXXVIII., 1882.

VOEGTLIN, C., and SMITH, H. W., 1920 [1], Quantitative studies in chemotherapy. I. Trypanocidal action of antimony compounds. *Ibid.*, XV.,453.

VOEGTLIN, C., and SMITH, H. W., 1920 [2], Quantitative studies in chemotherapy. II. The trypanocidal action of arsenic compounds. *Ibid.*, XV., 475.

VON JANCSÓ, N., and VON JANCSÓ, H., 1934, Mikrobiologische Grundlagen der chemotherapeutischen Wirkung. I Mitteilung. Wirkungsmechanismus des Germanins (Bayer 205) bei Trypanosomen. *Zbl. Bakt.* Abt. I Orig., CXXXII., 257.

YAMANOUCHI, T., 1910, Action de l'atoxyl sur les trypanosomiases dans l'organisme. *C.R. Soc. Biol., Paris*, LXVIII., 120.

YORKE, W., ADAMS, A.R.D., and MURGATROYD, F., 1929, Studies in chemotherapy, I. A method for maintaining pathogenic trypanosomes alive *in vitro* at 37° C. for twenty-four hours. *Ann. trop. Med. Parasit.*, XXIII., 501.

YORKE, W. and MURGATROYD, F., 1930, Studies in chemotherapy, III. The action *in vitro* of certain arsenical and antimonial compounds on *T. rhodesiense* and on atoxyl- and acriflavine-resistant strains of this parasite. *Ibid.*, XXIV., 449.

(2) The Rate of Absorption and Excretion of Trypanocidal Substances by the Tissues

The rate of conversion of arsenical compounds into trypanocidally active substances, the penetration of these substances into the tissues and their rate of excretion is obviously closely correlated with chemotherapeutic action. Thus, as Cohen, King and Strangeways (1932) point out, no apparent relation between the toxicity of arsonic acids for mice and the velocities of oxidation of the corresponding arsenoxides can be obtained, since it is masked by the rate of excretion (*cf.* also Voegtlin and Thompson, 1922). The excretion of quinquevalent arsenicals injected into the blood-stream has been studied in considerable detail by a number of observers, including Stühmer (1924), Rothermundt and Richter (1935), Richter (1937), and Launoy and Fleury (1937). The last observers found that when rabbits were injected with such a quantity of an 8 per cent. solution of tryparsamide that between 0·7 and 2·66 mgm. of arsenic were given per gm. of blood of the injected animal, doses well within the therapeutic range, from 88 to 95 per cent. of the arsenic injected had disappeared within an hour. Three to four hours after injection only about 1 per cent. of the injected arsenic could be discovered in the blood, and after six hours only 0·1 to 0·4 per cent. Imponderable traces, however, could still be found twenty-nine hours after injection provided from 10 to 15 c.c. of blood were examined. It seems probable that these persistent traces represent arsenic which has first been fixed by the tissues, converted into the trypanocidal tervalent form, and again slowly liberated by the tissues. This is the view put forward by Murgatroyd, Russell and Yorke (1934), who, instead of determining the blood content of various arsenicals in terms of arsenic, have studied the trypanocidal titre of the serum of rabbits after the intravenous injection of neoarsphenamine, reduced tryparsamide thioglycollate and tryparsamide, drugs which are examples of arsphenamines, arylthioarsinites and quinquevalent arsenicals.

It was found that injection of neoarsphenamine and reduced tryparsamide conferred immediately on the serum a very high trypanocidal titre, which is proportional to the dose of the drug injected. This titre immediately falls, at first quickly, and later more slowly until it reaches zero. The only difference in the two compounds is that the fall in titre in the case of the reduced tryparsamide is much more rapid than in the case of the arsphenamine compound. The immediate effect of injection of a quinquevalent compound is to confer only a slight trypanocidal titre on the serum. Instead of falling, however, as happens with the other two drugs, the titre steadily rises, and only attains its maximum six hours after the injection. The maximum titre reached is, moreover, in no way comparable to the enormous titres obtained with the arsphenamine and tervalent compounds. These two compounds obviously circulate unchanged and are rapidly eliminated from the blood-stream. Tryparsamide, on the other hand, must be reduced to the tervalent form before it becomes trypanocidal, and as soon as this happens, it is rapidly eliminated from the blood-stream. The trypanocidal activity and the arsenic content of the cerebrospinal fluid in man after the administration of arsenicals have been examined by Hawking, Hennelly and Quastel (1937). After the intravenous injection of 3 gm. of tryparsamide the trypanocidal activity of the cerebrospinal fluid in man, as tested by the action of the cerebrospinal fluid on trypanosomes *in vitro*, becomes apparent after fourteen hours, rises to a maximum after between thirty and forty hours and diminishes to such an extent as to be too small to measure after eighty hours. After injection of the same dose, the total arsenic content, measured chemically, is at a maximum after fourteen hours and falls off during the next forty hours, a result in agreement with Sicé, Cousin and Dantec (1933), who, after the last dose of a course of tryparsamide, detected traces of arsenic for from six to twelve hours after injection but not at later periods. The trypanocidal activity of the cerebrospinal fluid thus bears no definite relationship to the total amount of arsenic present, indicating that the ratio of arsenic existing in the trypanocidal (tervalent) form to the total arsenic present varies from patient to patient. The average values for the percentage of total arsenic present in the trypanocidally active form in the cerebrospinal fluid after intravenous injection of 3 gm. of tryparsamide are :—

After 14 hours	3 per cent.
After 40 hours	18 per cent.
After 60 hours	5 per cent.

The proportion of active arsenic in the cerebrospinal fluid thus depends on (i.) the rate of penetration of the original compound into the cerebrospinal fluid, (ii.) the rate of conversion of the quinquevalent into the tervalent form and the diffusion of the latter into the cerebrospinal fluid and (iii.) the rate of conversion of this active form into some inert substance by combination with tissue compounds. Orsanine (*cf.* p. 159) was equal or superior to tryparsamide in producing trypanocidal activity.

While neocryl (*cf.* p. 160) appears to be almost as active in the secondary stage of trypanosomiasis as tryparsamide, Hawking, Hennelly and Quastel (1937) were unable to determine the development of any trypanocidal activity in the cerebrospinal fluid, although neocryl undoubtedly penetrates into the central nervous system.

Intravenous injection of tervalent arsphenamines leads to the appearance of very low concentrations of arsenic in the cerebrospinal fluid, while the trypanocidal activity is slight or absent. This accords with the therapeutic inefficiency of these compounds in neurosyphilis and the secondary stage of sleeping sickness. These results are, however, in contradiction to those obtained by Cornwall, Bunker and Myers (1931), who found that arsenic penetrated into the cerebrospinal fluid in larger quantities after the intravenous injection of silver arsphenamine than after the injection of tryparsamide.

After intravenous injection of germanin into rabbits and dogs, the amount of the drug in the plasma has been shown by Dangerfield, Gaunt and Wormall (1938) to fall rapidly for the first few days, owing in part to excretion in the urine. After this initial period, diminution is less rapid, and five or six months after intravenous injection into a rabbit of 0·1 gm. per kilogram of body weight there is still present in the blood plasma at least 1 mgm. per 100 c.c. Several small injections of germanin given over a period of a few weeks cause a higher concentration of the drug in the plasma than a single injection of the same amount.

References

COHEN, A., KING, H., and STRANGEWAYS, W. I., 1932, Trypanocidal action and chemical constitution. Part XIV. The relative velocity of oxidation of arylarsenoxides. *J. Chem. Soc.*, 2866.

CORNWALL, L. H., BUNKER, H. A., Jr., and MYERS, C. N., 1931. Arsenic in spinal fluid: quantitative estimation following intravenous administration of tryparsamide and silver arsphenamine. *Arch. Neurol. Psychiat., London*, XXV., 137.

DANGERFIELD, W. G., GAUNT, W. E., and WORMALL, A., 1938, Studies on Bayer 205 (Germanin) and antrypol. I. The determination of small amounts of Bayer 205 (and antrypol); II. The persistence of Bayer 205 in the blood-stream after injection into animals. *Biochem. J.*, XXXII., 59.

Hawking, F., Hennelly, T. J., and Quastel, J. H., 1937, Trypanocidal activity and arsenic content of the cerebrospinal fluid after administration of arsenic compounds. *J. Pharmacol.*, **LIX.**, 157.

Launoy, L., and Fleury, O., 1937, Sur la vitesse d'élimination hors du courant sanguin, de l'arsenic injecté par voie veineuse, sous forme de tryparsamide, à des lapins normaux ou infectés par " Trypanosoma annamense." *Bull. Soc. Path. exot.*, **XXX.**, 315.

Murgatroyd, F., Russell, H., and Yorke, W., 1934, Studies in chemotherapy. XI. The trypanocidal titre of the serum of rabbits after the intravenous injection of various compounds of arsenic. *Ann. trop. Med. Parasit.*, **XXVIII.**, 227.

Richter, R., 1937, Zur Verteilung der Arsenobenzolderivate im Kaninchenorganismus. *Arch. Dermat. u. Sylph. Berl.*, **CLXXV.**, 239.

Rothermundt, M., and Richter, R., 1935, Untersuchungen über die Ausscheidung und Retention des Solu-Salvarsans im tierischen Körper. *Z. ImmunForsch.*, **LXXXV.**, 106.

Sicé, A., Cousin, E., and Dantec, 1933, Recherche de l'arsenic dans le liquide céphalo-rachidien des trypanosomés traités par la tryparsamide. *Bull. Soc. Path. exot.*, **XXVI.**, 1263.

Stühmer, 1924, Studien über die Primäraffektbildung beim Trypanosomen-Kaninchen (Primärkomplex, Infektion d'emblée, relativer Schutz des Erregers im Primäraffekt). *Arch. Dermat. u. Syph. Berl.*, **CXLV.**, 254.

Voegtlin, C., and Thompson, J. W., 1922, Quantitative studies in chemotherapy. VI. Rate of excretion of arsenicals, a factor governing toxicity and parasiticidal action. *J. Pharmacol.*, **XX.**, 91.

(3) The Action of Trypanocidal Drugs on Trypanosomes

It is now realised that in drug-resistant trypanosomes, the essential change is a decreased absorption of the drug by the parasite. Absorption of the drug by the trypanosome is thus seen to be the first essential for trypanocidal action. With certain trypanosomes the action of arsenicals can be explained by failure of absorption. Fischl and Singer (1935[2]), for instance, have pointed out that *T. lewisi* absorbs arsenophenylglycine which acts on the trypanosomes in considerable amount : the slightly active atoxyl is absorbed in much smaller amounts, while other aromatic arsenicals which do not kill *T. lewisi* are scarcely absorbed at all.

Although absorption of a certain quantity of the drug is necessary for curative effect, absorption is not synonymous with therapeutic action for a number of substances, solganal and sulfoharnstoff (both gold-containing compounds), atebrin and rivanol, which are totally devoid of trypanocidal action, are as readily absorbed by trypanosomes as the trypanocidal dye trypaflavine (Singer and Fischl, 1935 [2]).

The fact that atebrin, although it has a photodynamic action on the trypanosomes, is without lethal action disposes of the view that photodynamic action is an essential part of trypanocidal activity (Fischl and Singer, 1935 [1], Singer and Fischl, 1935 [1]), as does the fact that trypaflavine acts on trypanosomes in the dark.

So far as the quinquevalent arsenicals are concerned there is some evidence to show that absorption is greater when reduction has taken place to the arsenoxide. Thus Fischl and Singer (1935 [1]), for instance, found that when suspensions of *Spirochæta recurrentis*, *Proteus vulgaris*, erythrocytes, collodion and animal charcoal are placed for one hour in a solution of 0·1 per cent. atoxyl they absorb much less arsenic than when the atoxyl has previously been digested with liver. Glutathione was found to increase the absorption of atoxyl by trypanosomes. The amount of arsenic actually fixed by trypanosomes has been calculated by Reiner, Leonard and Chao (1932), who have estimated that if the volume of a trypanosome be taken as about 10^{-10} c.c. and its surface area as 10^{-6} sq. cm. the average amount of arsenic bound would be 0·1 microequivalents for 10^{10} trypanosomes. If each molecule of the phenylarsenious oxide type occupies 300 sq. A(3×10^{-4} sq. cm.) the surface of a single trypanosome should be occupied by 3×10^{7} molecules, arranged in a monomolecular layer. In fact this is five to ten times the number of molecules actually fixed, and hence the amount of arsenic absorbed is sufficient to cover only about one tenth of the surface of the trypanosome. If the trypanosomes are killed the amount of arsenic absorbed is of the order required to cover the surface of the trypanosome with a monomolecular layer.

When once absorption has occurred, and, in the case of a metallic compound, reduction or oxidation has produced the arsenoxide stage, a toxic action on certain substances in the body of the trypanosome follows. There is still, however, considerable uncertainty as to the mechanism by which the arsenic attacks trypanosomes. Ehrlich (1909) originally suggested that the toxic action of arsenicals might lie in their affinity for thiol groups. This suggestion was elaborated by Voegtlin, Dyer and Leonard (1923), who put forward the interesting hypothesis that, having arrived at the oxide stage, arsenic reacts with the reduced glutathione present in the tissues and the trypanosomes. In other words the —SH group forms the chemoreceptor for arsenic.

$$\mathrm{R.As = O + HS{-}R' \rightarrow R.As{\diagdown}S{-}R'}$$

In support of this view are the following facts :—

(1) By the nitroprusside reaction it is possible to show that trypanosomes contain an —SH group.

(2) If sodium thioglycollate is injected simultaneously with a minimum fatal dose of 3-amino-4-hydroxyphenylarsenoxide, a delay is noticed in the time of death of the animal.

(3) Feeding with glutamic acid and cysteine offers protection against a minimum fatal dose of 3-amino-4-hydroxyphenylarsenoxide administered three hours later.

(4) The injection of compounds containing an —SH group simultaneously with a trypanocidal drug slows the rate of disappearance of trypanosomes from an infected animal. Substances which act in this way include reduced glutathione, thioglycollic, thiolactic and thiosalicylic acids and cysteine.

The analogy between the chemotherapeutic interference previously described (*cf.* page 200) and the action of the —SH group will be obvious.

Much criticism has been directed against the view that the —SH group in trypanosomes represents the receptor for arsenic. Brown and Kolmer (1929), for instance, failed to establish any relation between the reduced glutathione content of animal organs and the liability of various species to arsenic intoxication. Smythe and Reiner (1933) compared the action on glutathione of arsenicals and sodium mono-iodoacetate, a substance believed to inactivate glutathione. The results were difficult to interpret on the simple hypothesis of a direct inactivation of glutathione, since neither cysteine nor a thiosulphate solution inhibited the action of the iodoacetate either *in vivo* or *in vitro*. Cohen, King and Strangeways (1931), however, as the result of the production of dithioarsinites by direct condensation of arylarsenious oxides with thiol compounds containing a carboxyl group, believe that there is justification for the hypothesis that the lethal action of arsenic on living tissues is a chemical action and may well be an action on thiol groups and possibly on glutathione in particular.

Nevertheless, glutathione acts as a coenzyme to glyoxylase. This fact is of interest, since if arsenicals act by interfering with glutathione they must at the same time affect the carbohydrate metabolism of trypanosomes. This metabolism is very intense in the case of pathogenic trypanosomes, except *T. cruzi*, since in twenty-four hours a trypanosome consumes twice its own weight of sugar whereas the energy requirement of a 70 kgm. man for the same period is contained in about 500 gm. of sugar. It is of interest to note that *T. cruzi*, which does not possess this intense activity, is not acted on by arsenicals, while the non-pathogenic ***T. lewisi***, which is also relatively unaffected by aromatic arsenicals, consumes only about a fifth of the sugar required by pathogenic, arsenic-susceptible trypanosomes. As Christophers and Fulton (1938 [1 & 2]) have shown, the oxygen uptake of trypanosomes is dependent on a plentiful supply of glucose and, in addition, certain drugs inhibit oxygen consumption, the effect being in some degree parallel to therapeutic action. Other observers have suggested either that arsenic combines with the iron of the respiratory pigment or that some essential enzyme is attacked. Arsenic, in high dilution, for instance, exerts a toxic action on liver lipase and other enzymes (*cf.* Clarke, 1937). In this connection certain observations of Quastel (1931) are of interest. While the free naphthylaminedisulphonic acids and their first *s*-carbamide derivatives are not trypanocidal and do not inactivate the enzyme fumarase, both actions begin at the second *s*-carbamide stage and are most marked at the third *s*-carbamide stage. A parallelism with the substantive properties of these derivatives to cotton fibres also exists. Some structure must therefore, apparently exist which is common to fumarase, trypanosomes, and cotton fibres, and this common factor must make for specific combination or absorption with the second and third *s*-carbamide derivatives of the naphthylaminedisulphonic acids. Glowazky (1937) also has shown that when exposed to the action of *para*-aminophenyl-arsenoxide, trypaflavin and sulfoharnstoff resistant trypanosomes exhibit a quantitative change of the aerobic and a qualitative change of the anaerobic sugar metabolism after only thirty minutes: in the case of atoxyl these changes are noticeable only after six hours' exposure.

So far as the arsenical compounds are concerned there is thus still uncertainty as to the particular enzymes of the trypanosomes that are attacked, although there is a considerable body of evidence to show that sulphur is of paramount importance in the pharmacodynamics of arsenic.

Even greater uncertainty still prevails in regard to the action of non-metallic compounds, more especially germanin. Von Jancsó and von Jancsó (1935), however, have suggested that germanin produces a toxic inhibition of the sugar metabolism of the trypanosomes and that in this condition of " sugar-hunger " the trypanosomes are more readily phagocytosed by the reticulo-endothelial system. Von Issekutz (1933) had previously shown that when infected animals are treated with germanin and their blood withdrawn a few hours later the contained trypanosomes exhibited diminished glycolysis *in vitro*.

References

Brown, H., and Kolmer, J. A., 1929, The relation of arsenicals to the glutathione content of animal tissues. *J. Pharmacol.*, XXV., 417.

Christophers, S. R., and Fulton, J. D., 1938 [1], Observations on the respiratory metabolism of malarial parasites and trypanosomes. *Ann. trop. Med. Parasit.*, XXXII., 43.

Christophers, S. R., and Fulton, J. D., 1938 [2], The inhibitive effect of drugs upon oxygen uptake of trypanosomes (*Trypanosoma rhodesiense*) and malaria parasites (*Plasmodium knowlesi*). *Ibid.*, XXXII., 77.

Clarke, A. J., 1937, General pharmacology in " Handbuch der experimentellen Pharmakologie," edited by A. Heffter, W. Heubner and J. Schuller. Berlin, IV., 208.

Cohen, A., King, H., and Strangeways, W. I., 1931, Trypanocidal action and chemical constitution. Part X., Arylthioarsinites. *J. Chem. Soc.*, 3043.

Ehrlich, P., 1909, Über den jetzigen Stand der Chemotherapie. *Ber. dtsch. chem. Ges.*, XLII., 17.

Fischl, V., and Singer, E., 1935 [1], Die Wirkungsweise chemotherapeutisch verwendeter Farbstoffe. *Z. Hyg. InfektKr.*, CXVI., 348.

Fischl, V., and Singer, E., 1935 [2], Die Chemotherapie der Rattentrypanose. *Ibid.*, CXVI., 652.

GLOWAZKY, F., 1937, Der Stoffwechsel der Trypanosomen unter der Einwirkung von Chemotherapeutica *in vivo*. *Ibid.*, **CXIX.**, 741.

QUASTEL, H., 1931, Trypanocidal action and toxicity to enzymes. *Biochem. J.*, **XXV.**, 1121.

REINER, L., LEONARD, C. S., and CHAO, S. S., 1932, Studies on the mechanism of chemotherapeutic action: binding of arsenicals by trypanosomes *in vitro*. *Arch. int. Pharmacodyn.*, **XLIII.**, 186.

SINGER, E., and FISCHL, V., 1935^1, Weitere Versuche ueber die Wirkung von Arzneimitteln *in vitro*. *Z. Hyg. InfektKr.*, **CXVI.**, 356.

SINGER, E., and FISCHL, V., 1935^2, Arzneifestigkeit und Chemikalein-gewöhnung der Trypanosomen. *Ibid.*, **CXVI.**, 683.

SMYTHE, C. V., and REINER, L., 1933, Toxic effect of sodium monoiodoacetate on trypanosomes. *Proc. Soc. exp. Biol., N.Y.*, **XXXI.**, 289.

VOEGTLIN, C., DYER, H. A., and LEONARD, C. S., 1923, On the mechanism of the action of arsenic upon protoplasm. *Publ. Hlth. Rep., Wash.*, **XXXVIII.**, 1882.

VON ISSEKUTZ, B., 1933, Beiträg zur Wirkung des Germanins. Mitteilungen I. und II. *Arch. exp. Path. Pharmak.*, **CLXXIII.**, 479, 499.

VON JANCSÓ, N., and VON JANCSÓ, H., 1935, Chemotherapeutische Mittel mit opsoninartiger Wirkung. *Z. ImmunForsch.*, **LXXXIV.**, 471.

(4) Specific Relationship between Arsenical Compounds and Certain Trypanosome Infections

It has been known for some years that all species of trypanosome do not react in the same way to the same aromatic arsenicals. *T. lewisi* of rats, for instance, is relatively insusceptible to those organic arsenicals which are more or less effective against *T. equiperdum*, *T. brucei* and other species of trypanosomes actively pathogenic to rats. These trypanosomes on the other hand are relatively unaffected by arsenophenylglycine, which is quite active against *T. lewisi*. A number of other compounds have been found by Kuhs and Tatum (1937) to be useless in infections due to *T. equiperdum* but active against *T. lewisi* infections in rats: these compounds are 4-carboxymethoxyphenylarsenoxide, 4-glycine-N-phenylarsonic acid, 5-amino-2-β-hydroxyethylamino-phenylarsonic acid, 3-amino-4-*n*-butylphenylarsonic acid, 4-arsonophenoxyacetic acid, 5-amino-2-N-amylaminophenyl-arsonic acid, γ-4-arsonophenoxypropanol, 3-amino-4-*n*-butyl-aminophenylarsonic acid, 4 - arsonophenoxyacetophenone, 4-β-hydroxy-*n*-propoxyphenylarsonic acid. On the other hand a few drugs are equally active in infections due both to *T. lewisi* and *T. equiperdum*. The nature of the side-chains and the chemotherapeutic indices of these three groups of compounds in infections due to *T. equiperdum*, *T. rhodesiense* and *T. lewisi*, respectively, are shown in the Table.

It will be noted that in the compounds effective on *T. lewisi* the majority possess carboxyl groups whereas in those effective on *T. equiperdum* there are no carboxyl groups but instead side-chains of a basic nature. Replacement of the OH radical of a carboxyl group by a radical such as the NH_2 or CH_3 changes the compound from effectiveness on *T. lewisi* to effectiveness on *T. equiperdum.*

NATURE OF SIDE-CHAINS AND CHEMOTHERAPEUTIC INDICES OF AROMATIC ARSENICALS IN INFECTIONS DUE TO

T. equiperdum, T. rhodesiense and T. lewisi.
(Kuhs and Tatum, 1937).

Group	Chemotherapeutic indices in infections due to :			Nature of side-chains	Position
	T. equiperdum	*T. rhodesiense*	*T. lewisi*		
I	8·7	—	0	$AsO_3H_2,OCH_2CH_2.OH$	1, 4
	10	—	0	AsO_3H_2,OCH_2COCH_3	1, 4
	12	6	0	$AsO_3H_2,NHCH_2.CONH_2$	1, 4
	18	—	0	$AsO_3H_2,NH_2NHC_2H_4OH$	1, 3, 4
	20	2	0	AsO,NH_2,OH	1, 3, 4
	25	4	0	AsO_3H_2,NHC_2H_4OH	1, 4
	30	—	0	$AsO_3H_2,NH_2,OCH_2CHOHCH_3$	1, 3. 4
	50	23	0	$AsO_3H_2,NH_2,OCH_2CH_2OH$	1, 3, 4
II	1·5	0	7	$As=As,2(NHCH_2COOH)$	1, 4, 4
	0	0	6	$AsO,OCH_2.COOH$	1, 4
	0	0	2	$AsO_3H_2,NHCH_2,COOH$	1, 4
	0	0	1·8	AsO_3H_2,NH_2,NHC_2H_4OH	1, 2, 5
	0	0	1·5	AsO_3H_2,NH_2,C_4H_9	1, 3, 4
	0	0	1·4	AsO_3H_2,OCH_2COOH	1, 4
	0	0	1·2	$AsO_3H_2,NH_2,NHC_5H_{11}$	1, 2. 5
III	1·1	0	2·5	$AsO_3H_2,OCH_2,CH_2CH_2OH$	1, 4
	1·0	0	1·1	AsO_3H_2,NH_2,NHC_4H_9	1, 3, 4
	1·6	0	2·2	$AsO_3H_2,OCH_2COC_6H_5$	1, 4
	8·3	0	1·3	$AsO_3H_2,OCH_2CHOHCH_3$	1, 4
IV	0	0	0	$AsO_3H_2,NHCH_2,COOH$	1, 2

The reason why certain compounds are effective in infections due both to *T. lewisi* and *T. equiperdum* is not apparent. Compounds effective in *T. lewisi* infections possess aliphatic side-chains which are acidic in nature, while those compounds curing *T. equiperdum* possess side-chains of a basic nature. A shift in the aliphatic side-chain from the ortho to the para position with respect to the arsenical radical produces a marked increase in therapeutic efficiency for *T. lewisi.*

Reference

KUHS, M. L., and TATUM, A. L., 1937, Specificity relationships between types of arsenicals and types of trypanosomes. *J. Pharmacol.*, LXI., 451.

(5) Synergic Action

The possibility of synergic action of two unrelated chemicals on pathogenic trypanosomes has been investigated both *in vitro* and *in vivo.* Von Jancsó and von Jancsó (1935) believe that germanin and trypaflavine exert a synergic action which they explain by the following experiments. Whereas a normal strain of *T. brucei* suspended in a solution of germanin for thirty minutes at 37° C. fails to absorb the drug, a strain of *T. brucei* systematically treated with trypaflavine gradually increases in permeability for germanin and when fully trypaflavine-resistant is rendered completely avirulent when exposed to germanin. Launoy (1935) found that orsanine in doses of 1 mgm. cured only about 23 per cent. of mice infected with nagana, while with a dose of 1·4 mgm. of lithium antimonylthiomalate only a very small percentage of mice were sterilised: when the two drugs were given together 70 per cent. of mice were cured. Similarly Browning and Gulbransen (1935) showed that tryparsamide and styryl 245, in doses which separately are only slightly active, when combined cure a high percentage of mice infected with *T. brucei.* This synergism may possibly be correlated with the fact that while tryparsamide is quickly absorbed and excreted the styryl compound is only slowly absorbed and acts very gradually. The trypanosomes may, therefore, be first damaged by tryparsamide and then acted on for a considerable period by the styryl compound.

References

BROWNING, C. H., and GULBRANSEN, R., 1935, Combined treatment of experimental trypanosome infections by chemotherapeutic agents. *J. Path. Bact.*, XL., 425.

LAUNOY, L., 1935, De l'action synergique de l'arsenic et de l'antimoine dans le traitement du nagana expérimental de la souris. *Bull. Soc. Path. exot.* XXVIII., 324.

VON JANCSÓ, N., and VON JANCSÓ, H., 1935, Chemotherapeutische Mittel mit opsoninartiger Wirkung. *Z. ImmunForsch.*, **LXXXIV.**, 471.

(6) Spontaneous Changes in the Reaction of Strains of Trypanosomes to Drugs

It has long been recognised that in man strains of *T. rhodesiense* are relatively resistant to the action of aromatic arsenicals, while recently isolated strains maintained in mice exhibit a similar resistance. On the other hand, after a number of passages in mice *T. rhodesiense* becomes highly sensitive to arsenicals. Repeated passage in guinea-pigs, however, does not lead to so rapid an increase in arsenic sensitivity. Strains of *T. brucei* may also exhibit a similar change in arsenic sensitivity. As pointed out by Browning and Gulbransen (1935), when recently introduced into, and of low virulence for mice, *T. brucei* is relatively resistant to various trypanocidal drugs, but when highly accommodated to mice and its pathogenicity increased to a maximum, infected mice are readily cured. Murgatroyd, Yorke and Corson (1937) have also found that changes occurred in the sensitivity to arsenicals of a strain of *T. brucei* as a result of five years' maintenance in the laboratory. In addition to increased sensitivity to arsenicals, mouse passage strains exhibited changes in morphology, increased pathogenicity for mice, decreased pathogenicity for guinea-pigs and loss of transmissibility by *Glossina morsitans*. The guinea-pig passage strain exhibited no change of morphology, a gradual increase in pathogenicity for guinea-pigs and mice and eventually a marked increase in sensitiveness to arsenicals, though this last change appeared more slowly than in the mouse passage strains. The guinea-pig strains likewise finally lost their capacity of being transmitted by *Glossina*, but in one strain this character persisted for 4 years, and so long as it remained transmissible by *Glossina* its original characters appeared to be preserved unchanged.

In connection with changes in the reaction of trypanosomes to drugs the observations of Broom, Brown and Hoare (1936) are of considerable interest. It was found that the electric charge of trypanosomes in laboratory animals is not constant but may be either positive or negative. These differences in the sign of the charge are apparently due to relapses having taken place in the previous history of the strain, since the charge of a relapse strain is the reverse of the parent strain. Since it was also found that positively charged trypanosomes are considerably more susceptible *in vivo* to tryparsamide than negatively charged variants of the same species, it follows that in estimating the chemotherapeutic index of drugs on trypanosomes the sign of the charge of the trypanosome must be taken into account. The sign of the charge can be determined by means of the salt concentration test devised by Broom, Brown and Hoare (1936).

References

BROOM, J. C., BROWN, H. C., and HOARE, C. A., 1936, Studies in micro-cataphoresis. II. The electric charge of hæmoflagellates. *Trans. R. Soc. trop. Med. Hyg.*, XXX., 87.

BROWNING, C. H., and GULBRANSEN, R., 1935, Variation in chemotherapeutic susceptibility associated with change in virulence of a strain of *Trypanosoma brucei*. *J. Hyg., Camb.*, XXXV., 180.

MURGATROYD, F., YORKE, W., and CORSON, J. F., 1937, Studies in chemotherapy. XIII. The changes observed in *T.brucei* as a result of five years' maintenance in the laboratory. *Ann. trop. Med. Parasit.*, XXXI., 145.

(7) The Rôle of the Reticulo-endothelial System in the Chemotherapy of Trypanosomiasis

The rôle of the reticulo-endothelial system in the chemotherapeutic action of trypanocidal drugs still remains somewhat problematical. Kritschewski (1927 $^{1 \& 2}$) claims that in mice splenectomy destroys the sterilising action of various trypanocidal drugs, while blockade of the reticulo-endothelial system does not. These results have been confirmed by Rubinstein (1928) in mice and by Lisgunova (1928) in rats. Kritschewski (1928) has further shown that loss of the spleen is compensated for if, in the splenectomised animal, the drug is injected in combination with agar, so as to form a depot from which slow absorption may occur. Hasskó (1931) believes that the decreased trypanocidal action of a number of chemotherapeutic agents in combined infection with trypanosomes and spirochætes is due to the fact that the latter damage the reticulo-endothelial system.

The chemotherapeutic action of germanin is also apparently closely bound up with an intact reticulo-endothelial system. Von Jancsó and von Jancsó (1934 and 1935), whose results have been confirmed by Schlossberger and Grillo (1935), believe that germanin opsonises the trypanosomes by interfering with their carbohydrate metabolism. The slightly damaged trypanosomes are thus rendered more fit for phagocytosis by the reticulo-endothelial cells of the liver, spleen and bone marrow.

Although germanin can produce a cure in mice that are splenectomised and have had their reticulo-endothelial cells blockaded by injections of a colloidal copper preparation, its chemotherapeutic index is then only 1 : 135 compared with 1 : 270 in the normal animal. In addition, in normal animals the time required for complete sterilisation of the blood is from fifteen to twenty-four hours, while in the animals with damaged reticulo-endothelial systems the time is from twenty-five to forty-four hours, the same period as is necessary to kill the trypanosomes *in vivo*. In the normal animal treated with germanin abnormal parasites are rarely seen in the blood because as soon as degeneration begins the parasites are removed from the circulation by phagocytosis : in the blockaded animal abnormal parasites are extremely common.

References

Hasskó, A., 1931, Experimentelle Untersuchungen über Misch- und Sekundärinfektion. II. Heilversuche bei mischinfizierten Tieren ; zugleich ein Beitrag zur Funktion des retikuloendothelialen Systems bei den chemotherapeutischen Heilungsvorgängen. *Zbl. Bakt.* Abt. I. Orig.. **CXXIII.**, **140.**

KRITSCHEWSKI, I. L., 1927[1], Über eine bisher noch unbekannte Funktion des retikulo-endothelialen Systems. I. Die Beziehungen der Chemotherapie und Chemoprophylaxie zum retikulo-endothelialen Apparat. *Z. Immun-Forsch.*, LIII., 506.

KRITSCHEWSKI, I. L., 1927[2], Das retikulo-endotheliale System und Chemotherapie. *Zbl. Bakt.*, Abt. I. Orig., CIV., 214.

KRITSCHEWSKI, I. L., 1928, Über noch unbekannte Funktion des retikulo-endothelialen Systems. V. Der Mechanismus der die Aktivität des chemotherapeutischen Effektes bestimmenden Funktion des retikulo-endothelialen Systems und ihre Unabhängigkeit von der Schutzfunktion. *Z. ImmunForsch.*, LIX., 1.

LISGUNOVA, A. W., 1928, Über noch unbekannte Funktion des retikulo-endothelialen Systems. IV. Die Beziehungen der Chemotherapie und der Chemoprophylaxis zum retikulo-endothelialen System der Ratten. *Ibid.*, LVII., 292.

RUBINSTEIN, P. L., 1928, Ueber eine bisher noch unbekannte Funktion des retikulo-endothelialen Systems. III. Die Abhängigkeit der Chemotherapie und Chemoprophylaxe von der Blockierung des retikulo-endothelialen Apparates. *Ibid.*, LVII., 107.

SCHLOSSBERGER, H., and GRILLO, J., 1935, Experimentelle Untersuchungen ueber Misch- und Sekundärinfektion. VI. Mitteilung : Weitere Versuche ueber den Einfluss einer Mischinfektion mit Rekurrens-spirochäten auf die trypanozide Wirkung des Germanins. *Zbl. Bakt.* Abt. I. Orig., CXXXV., 203.

VON JANCSÓ, N., and VON JANCSÓ, H., 1934, Mikrobiologische Grundlagen der chemotherapeutischen Wirkung. I. Mitteilung. Wirkungsmechanismus des Germanin (Bayer 205) bei Trypanosomen. *Ibid.*, Abt. I. Orig., CXXXII., 257.

VON JANCSÓ, N., and VON JANCSÓ, H., 1935, Chemotherapeutische Mittel mit opsoninartiger Wirkung. *Z. ImmunForsch.*, LXXXIV., 471.

THE EFFECT OF DRUGS ON TRYPANOSOMES IN TSETSE FLIES

A point of some interest is the question whether trypanosomes present in tsetse flies are affected by trypanocidal drugs present in the blood that the flies have taken up from animals or man. Roubaud (1910) fed *Glossina* infected with *T. dimorphon* on guinea-pigs treated with atoxyl and found that the intestine, but not the proboscis, of the flies was sterilised. Similarly, Rodhain and his collaborators (1913) could not sterilise the probosces of tsetse flies by feeding them on blood rendered trypanocidal by tartar emetic. Kleine and Fischer (1922), using Bayer 205, found that *Glossina* fed on animals treated with this drug were still infective. Duke (1927 and 1928) obtained similar results. Duke (1913) believed nevertheless that arsenophenylglycine would destroy *T. gambiense* in the intestinal tract of *G. palpalis*, though the trypanosomes already present in the salivary glands were unaffected.

The question has recently been studied by van Hoof, Henrard and Peel (1937). They find that a meal of blood containing tryparsamide or Bayer 205 does not destroy *T. gambiense* infecting the intestine, proventriculus and salivary glands of *Glossina*. Even the salivary gland trypanosomes found in the proboscis of infective *Glossina* do not appear to be affected. A limited experiment suggests that the same is true for neoarsphenamine and possibly for the antimonial Sdt. 411. Nevertheless, a considerable proportion of tsetse flies thus treated, although they harbour trypanosomes in the salivary glands and appear to be infective, are no longer able to transmit the infection to guinea-pigs.

The prolongation of the incubation period in guinea-pigs, bitten by tsetse treated in this manner, confirms the view that the drug modifies the virulence of the trypanosomes despite the absence of morphological changes. A preliminary meal on blood containing tryparsamide or germanin does not influence the evolution of *T. gambiense* in the tsetse, unless the drug is present in very large amounts. Such preliminary meals of drug-containing blood do not cause the trypanosomes to become drug-resistant. A preliminary feed on tryparsamide blood has no inhibitory effect on the development of tryparsamide-resistant trypanosomes in the tsetse, even though the dose of the drug given to the guinea-pig is appropriately large in relation to the degree of resistance of the trypanosome. Disinfecting meals given to tsetse flies during the course of the developmental cycle of the trypanosomes give results similar to those obtained with isolated infective flies. The frequency of infection is not lessened but transmission of the infection to guinea-pigs is delayed or inhibited. Analogous results were obtained with *T. brucei*, while *T. cazalboui* in tsetse flies given a germanin meal were notably less capable of causing infections. *T. congolense* appeared to escape entirely the effect of a drug meal.

References

DUKE, H. L., 1913, Some experiments with arsenphenylglycin and *Trypanosoma gambiense* in *Glossina palpalis*. *Proc. R. Soc., London*, LXXXVI., B, 19.

DUKE, H. L., 1927, Expériences sur la valeur du " Bayer 205 " en tant qu'agent prophylactique contre les trypanosomes se développant dans *G. palpalis*. *Rapport provisoire de la Com. intern. Soc. Nations pour l'Étude de la Maladie du Sommeil.*, p. 38, Geneva.

DUKE, H. L.. 1928, Étude de l'action du " Bayer 205 " sur le *T. gambiense* se développant chez *G. palpalis*. *Rapport final de la Com. intern. Soc. Nations pour l'Étude de la Trypan. hum.*, p. 111. Geneva.

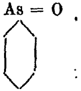
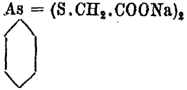
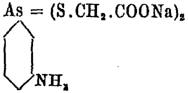
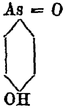
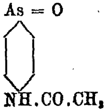
KLEINE, F. K., and FISCHER, W., 1922, Bericht über die Prüfung von Bayer 205 in Afrika. *Dtsch. med. Wschr.*, XLVIII., 1693.

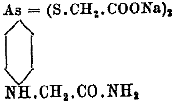
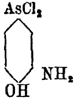
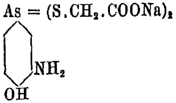
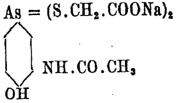
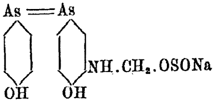
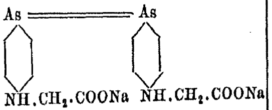
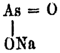
RODHAIN, J., VAN DEN BRANDEN, F., PONS, C., and BEQUAERT, J., 1913, Expériences et notes diverses. *Rapp. Miss. Sci. Katanga.*, p. 163. Bruxelles.

ROUBAUD, E., 1910, Précisions relatives aux phénomènes morphologiques du développement des trypanosomes chez les glossines. *C.R. Acad. Sci., Paris*, 1156.

VAN HOOF, L., HENRARD, C., and PEEL, E., 1937, Action de repas médicamenteux sur l'évolution des trypanosomes pathogènes chez la *Glossina palpalis*. *Ann. Soc. belge Méd. trop.*, XVII., 385.

THE RELATION BETWEEN THE RESISTANT FACTOR AND THE CHEMOTHERAPEUTIC INDEX FOR CERTAIN TERVALENT ARSENICAL COMPOUNDS (Hawking, 1937.)

Drug	Formula	Minimum trypanocidal concentration *in vitro* at 37° C. (Twenty-four hours)		Resistant factor	Chemo-therapeutic index	Lethal dose (mgm. per 20 gm. mouse)	Minimum effective dose (mgm. per 20 gm. mouse for normal strain)
		Normal Strain	*Resistant Strain*				
Phenylarsenoxide .	 $\mathrm{As = O}$	1 : 409,600,000	1 : 409,600,000	1	< 1	0·05	0·025
Phenylarsenoxide thioglycollate .	 $\mathrm{As = (S.CH_2.COONa)_2}$	1 : 204,800,000	1 : 204,800,000	1	< 1	0·1	0·05
Reduced atoxyl thioglycollate	 $\mathrm{As = (S.CH_2.COONa)_2}$ $\mathrm{NH_2}$	1 : 102,400,000	1: 25,600,000	4	2	0·15	0·075
p-Hydroxy-phenyl arsenoxide	 $\mathrm{As = O}$ OH	1 : 409,600,000	1 : 25,600,000	16	6	0·2	0·0375
Reduced arsacetin .	 $\mathrm{As = O}$ $\mathrm{NH.CO.CH_3}$	1 : 102,400,000	1 : 3,200,000	32	15	0·375	0·025

Reduced tryparsamide thioglycollate		1 : 102,400,000	1 : 200,000	512	100	3	0·03
Halarsol		1 : 102,400,000	1 : 3,200,000	32	50	1	0·02
Halarsol thioglycollate		1 : 102,400,000	1 : 3,200,000	32	50	1·5	0·03
Reduced stovarsol thioglycollate		1 : 6,400,000	1 : 400,000	16	7	1	0·15
Neoarsphenamine		1 : 51,200,000	1 1,600,000	32	40	5	(·125
Arseno-phenylglycine		1 : 12,800,000	1 : 6,400,000	2	4	2	0·5
Sodium arsenite	 $As=O$ $\vert$ ONa	1 : 3,200,000	1 : 3,200,000	1	< 1	0·175	0·15

CHAPTER VI

THE CHEMOTHERAPY OF SYPHILIS

THE therapeutic use of the arsenobenzene derivatives constitutes the greatest achievement yet made, not only in the treatment of syphilis but in the whole of chemotherapy. Arsphenamine was introduced in 1910 for the treatment of spirochætal infections by Ehrlich and Hata, and was first used by Wechselmann and Uhlenhuth and Mulzer for the treatment of syphilis in man, while neoarsphenamine appeared two years later. Despite this lapse of time, the arsenobenzene derivatives still hold pride of place as anti-syphilitics, though in bismuth has been found a useful adjuvant that has almost entirely replaced mercury. Arsphenamine, the dihydrochloride of 3 : 3′-diamino-4 : 4′-dihydroxyarsenobenzene, when dissolved in water is acid, and thus requires neutralization before use, when the monosodium salt is formed. With a slight excess of alkali, the disodium salt is produced, this being much less toxic than either the base or the monosodium salt. Owing to the inconvenience of having to dissolve the salt in water immediately before injection and the high percentage of reactions, the use of arsphenamine has been largely given up, despite the great efficiency of the compound. Neoarsphenamine, sulpharsphenamine and silver arsphenamine are now used in its place. The two former are official preparations of the British Pharmacopœia 1932.

Neoarsphenamine, which is sodium 3 : 3′-diamino-4 : 4′-dihydroxyarsenobenzene-N-methylenesulphoxylate, was prepared by Ehrlich (1912) by condensing the original arsphenamine with sodium formaldehyde sulphoxylate. The British Pharmacopœia (1932) demands that neoarsphenamine should contain about 20 per cent. of arsenic. Dyke and King (1934), in examining a number of commercial samples of neoarsphenamine, found that the percentage of arsenic varied from 17·8 to 21·6. A pure disubstituted methylenesulphoxylate of diaminodihydroxyarsenobenzene requires 26·5 per cent. of arsenic and a monosubstituted product 32·2 per cent. of arsenic. A study of the atomic ratios shows that the sulphur content of commercial neoarsphenamines in most cases approximates to that of a disubstituted derivative, but no accurate estimate of the molecular weight of neoarsphenamine can at present be given. Sulpharsphenamine consists mainly of disodium 3 : 3′-diamino-4 : 4′-dihydroxyarsenobenzene-N : N′-dimethylenebisulphite. It should be kept at a temperature below 15° C., and if it has become darker in colour should not be used.

While the choice of antisyphilitic drugs is still largely limited to arsenicals, as represented by the arsphenamines, and to bismuth, much work has been done on the methods of applying these medicaments.

The first essential for successful treatment is early diagnosis, since the chances of cure decrease in proportion as the age of the infection increases (Cannon, 1937) : in addition, as Moore (1936) insists, treatment must be prolonged for a period of at least twelve to eighteen months. Such treatment, consisting of arsenicals and a heavy metal, must either be continuous from start to finish, or the rest periods allowed must be so short that the patient continues to absorb either bismuth or mercury.

The best method of administering the arsenicals and heavy metals is still in dispute.

In Great Britain and in Denmark the usual rule in primary cases is to administer the arsenical and the metal simultaneously, this medication alternating with short periods during which no drug is given, the whole treatment occupying ninety weeks. In the United States of America continuous treatment, based on the extensive results of the Co-operative Clinical Studies, is employed, but with alternating courses of arsenic and bismuth, the period of treatment being sixty-five weeks. This alternation is preferred by Stokes (1937) since it is believed that it is the heavy metal, rather than the arsenical, which raises the reaction tendency of a system of treatment. However, as Martenstein (1935) points out, the intermittent system gives so much more treatment than the continuous system per unit of time that it compensates for, if it does not actually necessitate, rest periods. The various arguments for and against the continuous and intermittent treatment of early syphilis are ably discussed by Stokes and Usilton (1937), who state that if a patient with early syphilis has received at least thirty-two doses of an arsphenamine with an appropriate amount of heavy metal within a period of sixty-five weeks by the continuous method, or in a period of twenty-one months by the intermittent method, and is subsequently observed for from two to ten years, the incidence of relapse is 1·4 per cent. and 4·2 per cent. respectively. The continuous treatment system is also said to produce better serological results after three months than the intermittent system.

Adequate continuous and adequate intermittent systems are, however, both markedly superior to any irregular form of treatment and, if consistently carried out, serve to reduce to a minimum the involvement of the cardiovascular and nervous systems. The suggestion that the high incidence of cardiovascular syphilis met with in many large cities is due not so much to insufficient as to modern intensive methods of arsenical treatment, is contradicted by the fact that in Mongolia, where syphilis has been entirely untreated, the incidence of cardiovascular involvement is no less than in a city such as Berlin, where arsenicals and heavy metals are employed (Jessner and Rossiansky, 1930). In America, moreover, Cole *et al.* (1937) point out the correlation between inadequate treatment and the greater incidence of cardiovascular involvement in the negro as compared with the white.

THE STANDARDIZATION OF ARSPHENAMINE PREPARATIONS

In order to ensure that the arsphenamine preparations placed on the market while lacking in undue toxicity are nevertheless of a definite therapeutic value, certain tests have been proposed and, in accordance with the Memorandum of the International Conference on Biological Standards which met at Geneva in

September, 1925, the following compounds have been made the subject of internationally recognised standardisation for toxicity :

(1) Arsphenamine—the dihydrochloride of 3 : 3′-diamino-4 : 4′-dihydroxyarsenobenzene.
(2) Its metallic derivate silver arsphenamine.
(3) Its sodium salt.
(4) Neoarsphenamine—sodium 3 : 3′-diamino-4 : 4′-dihydroxyarsenobenzene-N-methylenesulphoxylate.
(5) Silver neoarsphenamine.
(6) Sulpharsphenamine.

Durham, Gaddum and Marchal (1929), as the result of extensive experiments, proposed a simplified procedure based on the use of the "integrated frequency" or "characteristic" curve for testing the toxicity of neoarsphenamine. This test has been slightly modified for official purposes (B.P., 1932), and is now as follows :—

(i) Ten mice, weighing 13 to 15 gm., are each injected intravenously with 0·3 c.c. of a 2 per cent. solution in freshly distilled water. Samples which do not cause the death of more than two mice can be passed without further testing.

(ii.) All samples giving a greater mortality in the first test than 20 per cent. are injected into a second batch of ten mice in the same dose. When the total mortality of both experiments is under 40 per cent., the sample is passed.

(iii.) Any sample which has killed more than eight but not more than fifteen mice in the first two tests, is injected into a further batch of ten mice. Samples which have killed not more than fifteen in all out of the total of thirty mice injected at the three stages, are passed, the others rejected.

A subsidiary test, described in the British Pharmacopœia, 1932, recommends the intravenous injection of a dose of 225 mgm. per kilogram of body weight of neoarsphenamine in a 5 per cent. aqueous solution into five rats. If not more than one rat dies the particular sample passes the test. In the official method prescribed by the Hygienic Laboratory of the United States Public Health Service, white rats weighing from 100 to 150 gm. are employed. For each toxicity test of arsphenamine not less than five rats are used, and at least 60 per cent. of the animals injected must survive at least forty-eight hours after injection of not less than 120 mgm. per kilogram of body weight. For neoarsphenamine, the rats must survive seven days after an injection of 240 mgm. per kilogram of body weight. The maximum tolerated dose of sulpharsphenamine, silver arsphenamine and silver neoarsphenamine, must be not less than 300, 140 and 180 mgm. per kilogram of body weight respectively.

The biological assay of sulpharsphenamine is similar to that for neoarsphenamine, except that sulpharsphenamine is given subcutaneously and not intravenously, and mice receive 0·35 c.c. of a 2 per cent. aqueous solution.

Although there is as yet no evidence of wide permanent differences in the response of strains of mice used in different European laboratories to the arsphenamines, such may have to be taken into account in laboratories in the tropics. Taylor and Ahuja (1935), for instance, found that white mice bred in Kasauli, India, from imported English stock, showed a susceptibility to the toxic action of neoarsphenamine which differed from that of the parent herd from which they were derived. A dose of 0·3 mgm. per gram of body weight in mice of 13 to 15 gm. weight gave with the Kasauli-bred mice a result comparable with that obtained by a dose of 0·43 mgm. in mice used for testing toxicity in England.

While individual rats from the same colony, as has been shown by Morrell and Chapman (1933 [2]), may exhibit considerable variations, not dependent on seasonal changes, in toxicity to neoarsphenamine, even more pronounced differences which appear to be constant may be found between albino rats derived from different colonies (Sampson and Latven, 1936). Whether these constant differences between animals derived from different colonies are dependent on hereditary or environmental factors is at present unknown. Wien (1936 [1 & 2]), however, showed that in mice fed on bread and milk the average lethal dose of neoarsphenamine was 10 per cent. higher than for mice fed on oats. Since lactose was a good substitute for whole milk, the enhanced value of the bread and milk diet was probably due to increased carbohydrate. A deficiency of vitamins A and D in the diet lowered the resistance of rats to neoarsphenamine approximately 17 per cent., but a deficiency of the vitamin B complex had much less effect.

In addition to variations in the susceptibility of the animals employed in testing the toxicity of arsphenamines, a number of observers (Rosenthal and Probey, 1933; Morrell and Chapman, 1933[1]; and Wien, 1935) have emphasized the importance of protecting solutions against oxidation by covering them with a layer of mineral oil. Unprotected 4 per cent. solutions of neoarsphenamine were found to increase in toxicity as much as 56 per cent. on standing for twenty minutes exposed to air, as compared with solutions five minutes old. Whether the increase in toxicity of solutions of arsphenamine with exposure to air is entirely due to increase in the amount of 3-amino-4-hydroxyphenylarsenious oxide, as suggested by Rosenthal and Probey (1933), is still uncertain. Schamberg, Kolmer and Johnson (1931) believe that arsenoxide is not the only factor.

According to Probey and Harrison (1938), the stability of neoarsphenamine is affected by the age of the product and by the moisture retained in the powder, the instability increasing as one or both of these influences are increased. Neoarsphenamine containing not more than 1·5 per cent. of volatile material may be safely kept for three years at temperatures below 20°C.

The correlation established by Dale *et al.* (1922) between trypanocidal activity in rats and spirochæticidal activity in rabbits has been reinvestigated by Schamberg and Kolmer (1933), who found that the results of trypanocidal tests coincided with the results of spirochæticidal tests in the case of fourteen out of seventeen different lots of neoarsphenamine. They suggest that in addition to a standard of minimal trypanocidal activity, 0·02 gm. of neoarsphenamine per kilogram of body weight should be taken as the minimal curative dose in acute rabbit syphilis, lymph node transfers from treated to normal rabbits being taken as criteria of cure.

References

Cannon, A. B., 1937, Optimal treatment for early syphilis, based on a twenty-year trial of arsphenamine, bismuth and mercury preparations. *Amer. J. Syph.*, XXI., 155.

COLE, H. N., USILTON, L. J., MOORE, J. E., O'LEARY, P. A., STOKES, J. H., WILE, U. J., PARRAN, T., and McMULLEN, J., 1937, Co-operative clinical studies in the treatment of syphilis. *J. Amer. med. Ass.*, **CVIII.**, 1861.

DALE, H. H., WHITE, C. F., BURN, J. H., DURHAM, F. M., MARCHAL, J. E., and MILLS, C. H., 1922, Report on an experimental and clinical comparison of the therapeutic properties of 914 (neosalvarsan). *Lancet, i.*, 779.

DURHAM, F. M., GADDUM, J. H., and MARCHAL, J. E., 1929, Reports on biological standards. II. Toxicity tests for novarsenobenzene (neosalvarsan). *Med. Res. Council Special Report* No. 128. London : H.M. Stationery Office.

DYKE, W. J. C., and KING, H., 1934, The constitution of neoarsphenamine. *J. Chem. Soc.*, 1707.

EHRLICH, P., 1912, Über Laboratiumsversuche und klinische Erprobung von Heilstoffen. *Chem. Ztg.*, **XXXVI.**, 637.

EHRLICH, P., and HATA, S., 1910, "Die experimentelle Chemotherapie der Spirillosen." Berlin : Julius Springer.

JESSNER, M., and ROSSIANSKY, N., 1930, Die Ergebnisse der deutsch-russischen Syphilis Expedition, 1928. *Arch. Derm. Syph., Wien.*, **CLX.**, 224.

MARTENSTEIN, H., 1935, Syphilis treatment ; enquiry in five countries. *Quart. Bull. Hlth. Org. League of Nations*, **IV.**, 129.

MOORE, J. E., 1936, The continuous method of treatment of early syphilis. *Ann. intern. Med.*, **X.**, 30.

MORRELL, C. A., and CHAPMAN, C. W., 1933[1], On the toxicity of neoarsphenamine. I. The increase in toxicity on exposure to air. *J. Pharmacol.*, **XLVIII.**, 375.

MORRELL, C. A., and CHAPMAN, C. W., 1933[2], On the determination of the toxicity of neoarsphenamine. II. The determination of the characteristic curve for rats. *Ibid.*, **XLVIII.**, 391.

PROBEY, T. F., and HARRISON, W. T., 1938, The effect of moisture and age on stability of neoarsphenamine. *Pub. Hlth. Rep.*, **LIII.**, 939.

ROSENTHAL, S. M., and PROBEY, T. F., 1933, The arsenoxide content and toxicity of fresh and old samples of arsphenamine. *J. Pharmacol.*, **XLI.**, 286.

SAMPSON, W. L., and LATVEN, A. R., 1936, Comparison of the toxicity of neoarsphenamine to different strains of rats. *J. Amer. pharm. Ass.*, **XXV.**, 1106.

SCHAMBERG, J. F., and KOLMER, J. A., 1933, Toxicity and physical properties of neoarsphenamine of different manufacture. *J. Amer. med. Ass.*, **C.**, 180.

SCHAMBERG, J. F., KOLMER, J. A., and JOHNSON, J. M., 1931, The influence of arsenicals and crystalline glutathione on the oxygen consumption of tissues. *Publ. Hlth. Rep. Wash.*, **XLVI.**, 339.

STOKES, J. H., 1937, Clinical problems in syphilis control to-day. *J. Amer. med. Ass.*, **CVIII.**, 780.

STOKES, J. H., and USILTON, L. J., 1937, Continuous and intermittent treatment for early syphilis : a critical review of the American and the League of Nations investigations with additional evaluations. *Arch. Derm. Syph., Wien*, **XXXV.**, 377.

TAYLOR, J., and AHUJA, M. L., 1935, Toxicity tests of novarsenobenzene in white mice bred in India. *Indian J. med. Res.*, **XXIII.**, 91.

UHLENHUTH, P., and MULZER, P., 1910, Die experimentellen Grundlagen chemotherapeutischer Versuche mit neueren Arsen-Präparaten. *Dtsch. med. Wschr.*, **XXXVI.**, 1262.

WECHSELMANN, W., 1910, Ueber die Behandlung der Syphilis mit Dioxy-diamino-arsenobenzol. *Berl. klin. Wschr.*, **XLVII.**, 1261.

Wien, R., 1935, Increase in toxicity for mice of solutions of neoarsphenamine on exposure to air. *Quart. J. Pharm.*, VIII., 631.

Wien, R., 1936[1], The influence of diet on the toxicity of mercurochrome and neoarsphenamine. *Ibid.*, IX., 48.

Wien, R., 1936[2], The influence of vitamin deficiency on the resistance of rats to neoarsphenamine, mercurochrome, pernocton and insulin. *Ibid.*, IX., 268.

TOXIC EFFECTS RESULTING FROM TREATMENT WITH ARSENOBENZENE DERIVATIVES

According to Cook and Wingo (1936), the total incidence of reactions to arsenobenzene derivatives, as judged by those occurring in the United States Navy in 1935, has shown little tendency to fall, although the ratio of fatal cases to injections shows improvement. Reactions for all drugs of the arsphenamine group amounted to approximately 1 per 1,000 injections, while the death rate was 1 per 37,101 injections of arsphenamine and 1 per 26,162 injections of neoarsphenamine. Phelps (1929) found that with arsphenamine the incidence of reactions was 1 per 683 injections, while the ratio of fatal reactions to doses was 1 to 17,769; in the case of neoarsphenamine the incidence of reactions was 1 per 1,109 injections, and the ratio of fatal reactions to doses 1 to 16,744.

As a result of an analysis of more than half a million doses of neoarsphenamine given in the United States Navy over a period of five years, Stephenson and Harrison (1938) find that the reaction expectancy increases directly with the age of the material injected.

There still exists considerable uncertainty as to the mechanism underlying the various reactions associated with the injection of arsenobenzene derivatives. Of the immediate reactions which include such symptoms as diarrhœa, vomiting, pyrexia, headache, medical shock, nitritoid crises and pulmonary embolism, the last, it is suggested, is due to the injection of solutions of arsphenamines the *p*H of which has passed to the acid side of neutrality with resulting precipitation of the arsphenamine. Acid arsphenamine shock is of short duration and is rapidly fatal, the symptoms being severe pain and oppression in the chest, paroxysmal coughing, circulatory collapse and death. Pathologically, numerous small emboli are found widely distributed, more especially in the lungs. Shivers (1933 and 1934) suggests that 0·8 mgm. of bromothymol blue should be added to each ampoule of the drug, so that the *p*H of each injection would be indicated.

The condition following the intravenous injection of neoarsphenamine termed "medical shock" was first clearly differentiated by Orr (1933), and has more recently been investigated by Weinberg (1937). The symptoms are extreme weakness, collapse and syncope ; the pulse is rapid, thready and may even be unobtainable. Nausea and vomiting are frequent ; the skin is cold and clammy, the face often cyanosed : the blood volume is diminished, the hæmoglobin and the erythrocyte count in the peripheral blood are increased. Sometimes there is complete anuria. Symptoms are not relieved by the injection of adrenalin ; in fact, in this type of shock vasoconstrictors may be dangerous, although in the typical nitritoid crisis adrenalin or ephedrine injections, as a rule, relieve the symptoms.

SENSITIZATION TO ARSPHENAMINE DERIVATIVES

The question whether these vasomotor crises, as well as certain forms of dermatitis and hæmorrhagic " encephalitis," are allergic in character is of considerable importance, since upon its solution depends an answer to the problem of whether pure chemical substances, other than proteins, can produce skin sensitization as well as true anaphylaxis.

It is now well recognised that certain persons apparently become sensitized as a result of injections of one of the arsphenamines ; as a rule, this sensitivity extends only to other arsenobenzene derivatives, but Epstein (1937[1]) has recorded six cases which were sensitive both to arsphenamines and to quinquevalent compounds such as tryparsamide.

On the one hand Birnbaum (1934), who gives a comprehensive review of the earlier literature, concludes that there is no proof that in human beings skin manifestations after the administration of arsphenamine are true allergic effects. He also casts doubt on the reports of passive transference of arsphenamine hypersensitiveness from one individual to another.

In the guinea-pig, however, numerous observers have succeeded in producing what appears to be true anaphylactic shock by injections of arsphenamine. Swift (1912) found that of nineteen guinea-pigs which were injected intraperitoneally or intravenously with neutralized arsphenamine mixed with guinea-pig serum, and reinjected after an interval, one died ten minutes and two others two hours after the injections, the symptoms resembling those of anaphylaxis. Other observers have also obtained fatal anaphylactic symptoms in guinea-pigs, very small amounts of neoarsphenamine being used as the sensitizing injections. Jadassohn and Schaaf (1934) found that the serum of sensitized guinea-pigs contained a hapten, the presence of which they believed was due to the fact that arsphenamine acted as a partial antigen. Working with the guinea-pig uterus by the Schultz-Dale method, Kallós and Kallós-Deffner (1935) found that, in the case of guinea-pigs subcutaneously injected with horse serum-arsphenamine mixtures, positive reactions were obtained in 7 out of 11 cases. Sensitivity could be passively transferred. Cormia (1937), however, failed to report these observations on the guinea-pig uterus. Lauf (1935) sensitized guinea-pigs by subcutaneous injections of neoarsphenamine and on giving intravenous injections, preferably six months later, obtained what appeared to be severe anaphylactic symptoms in approximately 15 per cent. of animals, while in some others the symptoms were slight. In no instance did death occur. The most convincing allergic phenomena, however, have been obtained by Landsteiner and Jacobs (1935 and 1936), who used unneutralized arsphenamine solutions. When the second injection was given intradermally, practically all the guinea-pigs showed large pink elevated areas in the centre of which necrosis frequently developed. When the second injection of arsphenamine was given intravenously, a large proportion of the animals showed symptoms suggestive of anaphylaxis and about half of them died with what appeared to be anaphylactic shock. With neoarsphenamine in place of arsphenamine the results were much less striking. The weight of experimental evidence is thus in favour of the view that under certain conditions true allergic manifestations can be produced by arsenobenzene derivatives. The results would seem to depend not only on the particular arsenobenzene derivative employed, but also on the physiological condition of the animal. Oser and Sulzberger (1934), for instance, claim that guinea-pigs treated with ascorbic acid show reduced sensitivity to neoarsphenamine, while Frei (1937) found that neither pregnant guinea-pigs nor young guinea-pigs under four weeks old can be sensitized to arsphenamine.

In man the occurrence of post-arsphenamine dermatitis is frequently precipitated by the presence of either focal or intercurrent infections (Stokes and Cathcart, 1923; and Stokes and Kulchar, 1934). On the other hand, injections of arsphenamine compounds may predispose to intercurrent or latent infections, such as furunculosis and erysipelas, zoster, herpes, lichen planus and molluscum contagiosum. Milian (1934) believes that the condition which he has termed "erythema of the ninth day" is an infection since it occurs not infrequently in epidemic form and is contagious (*cf.* Keim, 1935).

In view of the possible allergic nature of arsphenamine dermatitis the question has naturally been raised as to whether other arsphenamine reactions are allergic.

The view that the so-called hæmorrhagic encephalitis is allergic has much to commend it, the demyelinization, as pointed out by Russell (1937), being very similar to that seen in post-vaccinial and other forms of virus "encephalitides" that are probably of an allergic character.

DISORDERS OF THE LIVER ASSOCIATED WITH THE ADMINISTRATION OF ARSPHENAMINE PREPARATIONS

It has long been recognized that jaundice, both mild and severe, can occur during the course of syphilis. Gruner, in his book "Aphrodisiacus sive de lue venerea," published at Jena in 1789, quotes an observation attributed to Paracelsus, "Icterus cum morbo gallico copulatus non curatur nisi subacuta materia venerea," and occasionally jaundice was associated with the administration of mercury (*cf.* Gubler, 1853). It was, however, only with the advent of arsphenamine medication that the occurrence of jaundice became a well-recognized phenomenon.

The incidence of jaundice in association with arsphenamine injections, either intratherapeutic or post-therapeutic, shows very considerable variation in different countries and in different clinics, as is seen in the Table. The incidence would seem to be higher in France than in either Great Britain or the United States of America, but lower than either in India, where, according to Rajam (1935), the percentage incidence is only 0·29, despite the fact that neoarsphenamine is not well tolerated by Indians, dermatitis occurring in 1 of every 126 patients. No correlation can be found between jaundice and either intermittent or continuous treatment. Jaundice has been associated with all the arsphenamine preparations, but is less frequent with neoarsphenamine than with arsphenamine: thus among 12,177 patients receiving arsphenamine, 259 (2·12 per cent.) suffered from jaundice, while among 6,027 treated with neoarsphenamine, 78 (1·29 per cent.) developed this symptom (Medical Research Council, 1922, Special Report No. 66). While comparatively rare after tryparsamide and bismuth, both Wilcox (1933) and Graffar (1937) have recorded instances where those drugs were the only antisyphilitic medicaments employed.

Cole *et al.* (1931) found that females are more sensitive to arsenicals than males, and white more sensitive than coloured females. Snodgrass and Peters (1937), however, reported an incidence twice as great in males as in females. Pregnancy does not appear to predispose to the onset of jaundice. A curious fact noted by many observers is the irregular incidence of jaundice in the same clinic and with the same methods of administration. Stokes, Ruedemann and Lemon (1920), for instance, observed 70 cases at the Mayo Clinic in the four years from August 1916 to July 1920. Of these 70 cases no less than 64 occurred between October 1919 and April 1920: 41 per cent. of these patients developed an acute upper respiratory infection either just before or in association with the onset of the icterus. Bodin (1921) obtained similar figures. Of 254 persons treated with neoarsphenamine between 1912 and 1914, 2, or 0·78 per cent., developed jaundice: of 113 persons treated during 1919 and 1920, 2, or 1·77 per cent., but of 472 persons treated in 1921, 34, or 7 per cent., suffered from jaundice. Ruge (1932), who studied the incidence of jaundice in the German Navy from 1919 to 1929, noted a sudden increase in common infective hepatic jaundice unassociated with antisyphilitic treatment in 1921 : arsphenamine jaundice also increased in frequency and reached a peak a little later. In India, Rajam (1935) observed 20 patients with jaundice in three years : more than half of these were seen in one year. The incidence was specially high in the rainy season.

Soffer (1937), in discussing the frequency of jaundice in patients treated with arsphenamines in Baltimore, noted a gradual rise from 1919 to 1923–25, a fall to a low level in 1933, followed by a second sudden increase in 1934.

Graffar (1937), in Belgium, found that for the thirteen years 1919 to 1932 the highest incidence was from 1920 to 1921.

ANTISYPHILITIC TREATMENT AND JAUNDICE

No. of patients receiving anti-syphilitic treatment.	No. of cases of jaundice.	Percentage of cases of jaundice.	Country.	Reference.
10,000	84	0·84	Gt. Britain	Harrison (1916).
28,204	337	1·19	,, ,,	Med. Res. Council (1922).
570	23	4·0	,, ,,	Snodgrass and Peters (1937).
1,100	55	5·0	France	Clément-Simon and Vulliémoz (1920).
839	38	4·5	,,	Bodin (1921).
1,369	103	7·5	,,	Filliol (1929).
4,059	246	6·0	Belgium	Graffar (1937).
5,200	70	1·3	U.S.A.	Stokes, Ruedemann and Lemon (1920).
1,212	20	1·65	,,	Cole *et al.* (1931).
2,100	12	0·57	,,	Ireland (1932).
4,126	56	1·36	,,	Wile and Sams (1934).
18,250	158	0·87	,,	Soffer (1937).
7,160	21	0·29	India	Rajam (1935).

This variation in the incidence of arsphenamine jaundice, apart from differences in treatment, suggests that some factor other than the drugs is necessary for the incidence of arsphenamine jaundice.

The total amount of arsenical drug administered appears to play no part in the development of jaundice. The most frequent time of onset, however, is during, or shortly after the completion of, the first ten injections. According to Soffer (1937), in 76 per cent. of cases the jaundice appeared within ten days of the last injection of arsphenamine; on the other hand, the interval before the onset of jaundice has been as long as 280 days after the last injection of arsenic.

The symptoms of jaundice are malaise, nausea, vomiting and pain over the liver : fever may be present. In all except cases of acute hepatic necrosis, symptoms disappear within ten days of the onset of icterus, but jaundice may last for as long as 100 days. Slight secondary anæmia is present in about half the patients. The majority of affected persons appear to recover completely (*cf.* Biekind, Epstein and Kerr, 1934), but Campbell and Soffer (1937) found that 37 per cent. of patients who had had arsphenamine jaundice six to fourteen years previously, exhibited an abnormal degree of bilirubin retention. A similar result was found in 16 per cent. of syphilitics who had received arsphenamines but had never suffered from jaundice.

It seems possible, however, that antisyphilitic treatment may occasionally be responsible for toxic cirrhosis (Baldridge, 1934) as it is responsible also for acute and subacute necrosis of the liver. The incidence of liver necrosis is somewhat curious, for acute necrosis not infrequently occurs in epidemic form. At Ingolstadt, in Germany, Silbergleit and Föckler (1919) recorded that twenty-one syphilitic soldiers treated with arsphenamine developed symptoms of liver necrosis ; thirteen of the patients died. During 1917 a similar outbreak occurring at Cherry Hinton Military Hospital, Cambridge, resulted in the death of fifteen patients with acute liver necrosis.

A number of theories has been advanced to account for the association of jaundice with antisyphilitic medication, of which the following are the most important :—

(1) Jaundice is due to a syphilitic hepatitis.

(2) Jaundice is due to the action of arsphenamine on the liver.

(3) Jaundice is due to allergy and is a Herxheimer phenomenon.

(4) Jaundice is due to intercurrent infection with the agent of common infective hepatic jaundice (epidemic catarrhal jaundice).

Syphilis may undoubtedly attack the liver. O'Leary, Green and Rowntree (1929) distinguish acute syphilitic hepatitis, either benign or severe, from late syphilitic hepatitis which may be asymptomatic, diffuse or gummatous. Nevertheless the incidence among untreated syphilitics of actual jaundice is very low, probably no greater than among the ordinary non-syphilitic population. Werner (1897), among 15,799 patients with untreated early syphilis, observed a percentage incidence of jaundice of 0·37, Goldstein (1897), among 7,462 similar patients, an incidence of 0·26 per cent., while Wile and Sams (1934) found only 15 cases of jaundice (0·14 per cent.) among 10,021 untreated patients. Irgang (1937), however, found that the liver was in a state of mild dysfunction in 17 per cent. of 118 patients with secondary syphilis and in 24 per cent. of 50 patients with cutaneous manifestations of tertiary syphilis. In fatal cases of acute necrosis of the liver associated with arsphenamine jaundice spirochætes are not as a rule found in the liver (Graffar, 1937). The possibility that jaundice is due to a hepato-recurrence has received some support from the work of Milian (1920), who claims to have shortened greatly the incidence of arsphenamine jaundice by continuing the administration of arsphenamine: Milian's results, however, have not been confirmed. The fact that jaundice may occur when the Wassermann reaction is negative, is also in opposition to the theory that jaundice is due to syphilis alone.

The arsphenamines, if given in sufficiently large doses and even in therapeutic doses (*cf.* Soffer, 1936), may damage the liver. The liver is in fact the chief organ removing arsphenamines from the blood (Kraft *et al.*, 1938). Sager (1936), in fact, believes that the arsphenamines are themselves sufficiently hepato-toxic to account for post-arsphenamine jaundice. In therapeutic doses the arsphenamines if combined with other hepato-toxins may exert a deleterious effect on liver cells. Findlay, Dunlop and Brown (1931), for instance, found that in rabbits therapeutic doses of neoarsphenamine combined with the injection of a non-pathogenic strain of *Bacterium coli* caused much more extensive damage to the liver parenchyma than either the neoarsphenamine or *Bact. coli* alone. Masson (1925) also showed that if the liver is previously damaged by chloroform the proportion of a subsequent dose of arsphenamine taken up by the liver cells is greater than normal. In this connection Lewin (1933) has found that the excretion of bile salts is increased by administration of the arsphenamines, more especially in patients who are also suffering from infections of the upper respiratory tract. Lamb and Blakely (1937) have also shown that injections of neoarsphenamine increase the phosphatase content of the serum. Thus, despite the fact that arsenic cannot be found in the urine or stools of icteric patients, and though in the majority of syphilitic patients the administration of arsphenamines is followed by an improvement in the condition of the liver, as shown by liver function tests (Kellogg, Epstein and Kerr, 1936), it is possible that the onset of jaundice may in certain instances be precipitated by the action of some trivial intercurrent infection on a liver slightly damaged by arsphenamine.

The suggestion that the occurrence of jaundice is always a phenomenon of the Herxheimer type, the focal reaction producing congestion of the liver and jaundice is unlikely, in view of the fact that jaundice is not commonly associated with dermatitis or other allergic symptoms, while after the subsidence of jaundice, arsphenamine therapy may safely be resumed, often within a period as short as thirty days. The possibility that arsphenamine jaundice is in reality epidemic catarrhal jaundice has been suggested by a number of observers. It is now recognized that the disease long known as epidemic catarrhal jaundice is in reality a hepatitis and is distinguishable, as Hurst and Simpson (1934) point out, from the rarer condition of true catarrhal jaundice in which the mucosa of the duodenum is the site of an inflammatory condition. The term " common infective hepatic jaundice " is, therefore, preferable to epidemic catarrhal jaundice. Although common infective hepatic jaundice is highly infectious to man and has an incubation period of about four weeks, all attempts up to the present to transmit the disease to laboratory animals have failed. The causal agent, although probably a virus, is thus unknown, and proof of the identity of arsphenamine jaundice and common infective hepatic jaundice is consequently impossible. Nevertheless, there are certain facts which suggest that the conditions are very similar.

(1) The clinical symptoms are identical. Attempts to distinguish the diseases on such symptoms as the degree of fever and abdominal discomfort are impossible. Lerman (1929) believes that in arsphenamine jaundice the blood count exhibits a leucopenia, with relative lymphocytosis less frequently than in common infective hepatic jaundice. It must, however, be remembered that in arsphenamine jaundice the bone marrow may be stimulated by the presence of arsenic.

(2) Reference has already been made to the occurrence of arsphenamine jaundice in small epidemics in clinics where the method of administration of arsphenamines has been unchanged for many years.

(3) Arsphenamine jaundice appears to be infective, and instances where jaundice has been transmitted from patients receiving arsphenamine to non-syphilitics have been reported by Chevallier and Paillard (1920), Cerf and Bernard (1922) and Graffar (1937). Slosse (1921) records that in one clinic the medical personnel suffered from attacks of jaundice in all respects similar to those experienced by those receiving antisyphilitic treatment. Richards (1933) describes an outbreak in which there seems little doubt that infection was transmitted by contact. From 1931 onwards jaundice appeared in a clinic in patients receiving antisyphilitic treatment, and finally became so frequent that one in every three patients developed jaundice. In all, over 120 cases were seen. One patient developed jaundice who had not received arsenical treatment for over a year, while another had received only bismuth. Female patients were relatively immune. This was explained when it was realized that the male patients were forced to wait in a crowded and badly ventilated corridor. When excessive crowding was avoided and all patients were made to wait in a large well-ventilated out-patient hall, the incidence of jaundice immediately fell.

(4) Outbreaks of jaundice have been reported after the administration of other than antisyphilitic drugs. Murray (1930), for instance, reported that of patients in one clinic treated with acriflavine for gonorrhœa, no less than 11 per cent. developed symptoms indistinguishable from those of infective hepatic

jaundice eight or more weeks after cessation of treatment. Although certain cases of arsphenamine jaundice may be associated with the direct toxic action of arsenic on the liver, or with hepato-recurrence drug sensitivity, it seems not impossible that injections of arsphenamine may predispose to infection with the agent of common infective hepatic jaundice, much in the same way as injections of arsenic may predispose to infection with the virus of herpes zoster.

THE PREVENTION AND TREATMENT OF REACTIONS DUE TO ARSPHENAMINE DERIVATIVES

The prevention of reactions due to arsenobenzene derivatives may be divided into measures taken (1) to determine in which persons reactions are likely to occur and (2) to reduce the toxicity of the drugs.

Various tests have been proposed in order to determine whether patients are sensitive to arsphenamine preparations. Schreiner and Ensbruner (1934) have proposed a complement fixation reaction, but the test most commonly employed has been either the intradermal injection of one of the arsphenamine compounds or the " patch " test originated by Jadassohn (*cf.* Jadassohn and Schaaf, 1934), in which a pledget of material soaked in the drug is applied to the skin. The general view now held is that the patch test is not sufficiently specific to be relied on implicitly. Cannon and Karelitz (1934), Robinson (1934 and 1936) and Beerman (1936) believe that while a strongly positive reaction in a person who has suffered from dermatitis precludes the further administration of any of the arsphenamines, a weakly positive or negative test should be followed up at least three months later by an intravenous injection of not more than 0·25 gm. of a different member of the arsphenamine group from that originally inducing the dermatitis. If no reaction occurs within a week, the dose may be doubled. Indiscriminate use of the patch test may not be entirely harmless, since it may cause an exacerbation of a subsiding dermatitis or, if repeated too often, induce cutaneous sensitization to the particular arsphenamine employed.

For the prevention of acute hepatitis following arsphenamine, dextrose has been recommended by Shaffer (1934), though if post-arsphenamine hepatitis is really an infection its efficacy is doubtful. Urine examinations for urobilinogen and bile salts are possibly also of value in the early recognition of cases that are liable to develop serious reactions.

In the prevention of immediate reactions, more especially early nausea and vomiting and the nitritoid crisis, a very considerable number of drugs have been recommended, including preliminary treatment with sodium bicarbonate, sodium iodide, calcium salts, cæsium eosinate, strontium bromide, vitamin C and atropine. The administration of adrenalin, either five minutes before the injection of the arsphenamine or at the same time, has been suggested, as has the very slow administration of the drug, the injection of one-tenth of the dose followed forty-five minutes later by the remainder of the drug, or the administration of either acid or alkaline sodium phosphate for one week before the injection. Snodgrass and Peters (1937) find that very slow administration, heavy purgation twenty-four hours before the injection, and the injection of 0·25 to 0·5 c.c. of adrenalin 1 in 1,000, five minutes before the arsphenamine, are of value.

In order to decrease the toxicity of solutions of arsphenamine preparations, attempts have been made to combine the arsenicals with crude liver extract (Villaret, Justin-Besançon and Desoille, 1937; and Galliot, 1937), with amino-acids obtained from the liver (Maderna, 1934; and Rebaudi, 1934), with glycocoll (Spillman and Krumholz, 1936; Lambert, 1937), or stabisol, a sodium benzoate methenamine preparation (Benetazzo, 1937). If neoarsphenamine is dissolved in a 4 per cent. solution of glycocoll, 0·15 gm. of neoarsphenamine being dissolved in 1 to 2 c.c. of the glycocoll solution, it is claimed that the toxicity is decreased so that greater amounts of arsphenamine may be injected in a given time, while treatment may be resumed in persons who are normally intolerant to arsphenamine preparations. Durel (1936) found that experimentally there was some slight evidence in support of these claims, for the addition of an amount of glycocoll equal to that of neoarsphenamine did not affect the therapeutic activity of the drug as determined by its effect on mice infected with nagana: the toxicity was, however, reduced by about 10 per cent.

Craven (1931) believes that high fat and high protein diets provide maximum protection against liver injury caused by arsphenamine, the former being the more efficient. High sugar diets and starvation predispose to the liver injury caused by arsphenamine.

Appel (1933) and Appel and Jankelson (1935) believe that if each dose of arsphenamine is mixed with 10 c.c. of a 5 per cent. solution of sodium dehydrocholate and injected very slowly intravenously, it is possible to continue arsphenamine medication even when an acute hepatitis is present, provided bile is still present in the stools, since the dehydrocholate increases the amount of arsenic excreted in the bile.

Apart from the use of sodium dehydrocholate the treatment of arsphenamine hepatitis is similar to that of other forms of acute toxic hepatitis and consists of the injection of hypertonic glucose solution, insulin and calcium gluconate.

A similar treatment has been used for arsenical dermatitis by Shaffer (1934) and Epstein (1937 [2]) with the addition of either sodium or calcium thiosulphate (*cf.* McLachlan, 1936). Apart from its alleged curative effects in other allergic conditions, the reason for the use of sodium thiosulphate, introduced by Ravaut (1920), is still uncertain, for while Mattice and Weisman (1937) have claimed that, so far from increasing the urinary excretion of arsenic, the administration of sodium thiosulphate actually leads to a decreased excretion for a period of some days, Ayres and Anderson (1938) have found the exact opposite.

References

APPEL, B., 1933, Sodium dehydrocholate in arsphenamine poisoning. *Arch. Derm. Syph., N.Y.*, XXVII., 401.

APPEL, B., and JANKELSON, I. R., 1935, Treatment of arsenical hepatitis with sodium dehydrocholate. *Ibid.*, XXXV., 422.

AYRES, JR. S., and ANDERSON, N. P., 1938, Sodium thiosulfate and the elimination of arsenic. *J. Amer. med. Ass.*, CX., 886.

BALDRIDGE, C. W., 1934, Relationship between antisyphilitic treatment and toxic cirrhosis. *Amer. J. med. Sci.*, CLXXXVIII., 685.

BEERMAN, H., 1936, The patch test in the determination of arsphenamine sensitization. *Penn. med. J.*, XXXIX., 690.

BENETAZZO, G., 1937, Tentativi di attenuazione degli inconvenienti tossici degli arsenobenzoli. *G. ital. Derm. sif.*, LXXVIII., 495.

BIEKIND, G. R., EPSTEIN, N. N., and KERR, W. J., 1934, Hepatic complications in the treatment of syphilis. The Rose Bengal test as a means of detecting disturbances of liver function and its rise as a guide in the therapy of syphilis. *Ann. intern. Med.*, VII., 966.

BIRNBAUM, G., 1934, Neuere Untersuchungen über die Salvarsanschädigungen der Haut. (Kritischer Ergebnisbericht). *Zbl. Haut-u. GeschlKr.*, XLIX., 97.

BODIN, E., 1921, Les ictères au cours du traitement de la syphilis par le novarsénobenzol. *Bull. Soc. franç. Derm. Syph.*, XXVIII., 242.

CAMPBELL, A. D., and SOFFER, L. J., 1937, The results of the bilirubin test for liver function on patients recovered from arsphenamine jaundice. *Amer. J. Syph.*, XXI., 420.

CANNON, A. B., and KARELITZ, M. B., 1934, Intradermal tests in relation to arsphenamine dermatitis. *Arch. Derm. Syph., N.Y.*, XXIX., 485.

CERF, M., and BERNARD, R., 1922, Contribution à l'étude des ictères survenant au cours de l'évolution et du traitement de la syphilis. *Brux. méd.*, II., 556.

CHEVALLIER, P., and PAILLARD, H., 1920, L'ictère postarsénobenzolique des syphilitiques. *J. méd. franç.*, IX., 64.

CLÉMENT-SIMON and VULLIÉMOZ, 1920, Note sur 55 cas d'ictère observés en un an chez 1,100 syphilitiques traités par le novarsénic. *Bull. Soc. franç. Derm. Syph.*, XXVII., 263.

COLE, H. N., DEWOLF, H., MCCUSKEY, J. M., MISKJIAN, H. G., WILLIAMSON, G. S., RAUSCHKOLB, J. R., RUCH, R. O., and CLARK, T., 1931, Toxic effects following use of the arsphenamines. *J. Amer. med. Ass.*, XCVII., 897.

COOK, S. S., and WINGO, E. H., 1936, Toxic effects of arsenical compounds as administered in the United States Navy in 1935 with special reference to arsenical dermatitis. *Nav. med. Bull., Wash.*, XXXIV., 569.

CORMIA, F. E., 1937, Experimental arsphenamine dermatitis. Observations on its allergic nature with special attention to the Schultz-Dale phenomenon. *Arch. Derm. Syph., N.Y.*, XXXVI., 970.

CRAVEN, E. B., 1931, Importance of diet in preventing acute yellow atrophy during arsphenamine treatment. *Johns. Hopk. Hosp. Bull.*, XLVIII., 131.

DUREL, P., 1936, Influence du glycocolle sur la toxicité et l'activité du novarsénobenzol chez la souris. *Bull. Soc. franç. Derm. Syph.*, XLIII., 1818.

EPSTEIN, E., 1937 [1], Sensitivity to both trivalent and pentavalent arsenicals. *Arch. Derm. Syph., N.Y.*, XXXVI., 964.

EPSTEIN, E., 1937 [2], Postarsphenamine exfoliative dermatitis: etiology, complications and treatment. *J. Amer. med. Ass.*, CIX., 117.

FILLIOL, L., 1929, Contribution à l'étude des ictères parathérapeutiques. *Thèse de Paris.*

FINDLAY, G. M., DUNLOP, J. L., and BROWN, H. C., 1931, Observations on epidemic catarrhal jaundice. *Trans. R. Soc. trop. Med. Hyg.*, XXV., 7.

FREI, W., 1937, Tierexperimenteller Beitrag zur Frage "Salvarsan und Säugling." *Acta derm.-venereol.*, XVIII., 396.

GALLIOT, A., 1937, Le foie et les traitements antisyphilitiques. *Ann. Mal. vén.*, XXXII., 329.

GOLDSTEIN, O., 1897, Beiträge zur Pathologie des Ikterus syphiliticus. *Münch. med. Wschr.*, XLIV., 736.

GRAFFAR, M., 1937, Contribution à l'étude des ictères survenant au cours du traitement arsénical. *Pr. méd.*, XLV., 661.

GUBLER, 1853, Mémoire sur l'ictère qui accompagne parfois les éruptions syphilitiques précoces. *C. R. Soc. Biol., Paris*, V., 225.

HARRISON, L. W., 1916, The treatment of syphilis. *Quart. J. Med.*, X., 291.

HURST, A. F., and SIMPSON, C. K., 1934, Catarrhal jaundice and mild hepatic necrosis: their pathology and diagnosis. *Guy's Hosp. Rep.*, LXXXIV., 173.

IRELAND, F. A., 1932, Reaction following the administration of the arsphenamines and a method of prevention. *Amer. J. Syph.*, XVI., 22.

IRGANG, S., 1937, The problem of involvement of the liver in syphilis: comment on its more important phases, with case reports. *Arch. Derm. Syph., N.Y.*, XXXVI., 685.

JADASSOHN, W., and SCHAAF, F., 1934, Untersuchungen über den Mechanismus der anaphylaktisch-urtikariellen Reaktion. (Schultz-Dalesche Versuche mit Haptenen.) *Arch. Derm. Syph., Wien*, CLXX., 33.

KALLÓS, P., and KALLÓS-DEFFNER, L., 1935, Experimentelle Untersuchungen über Salvarsanallergie. *Klin. Wschr.*, XIV., 1074.

KEIM, H. L., 1935, Erythema of the ninth day. *Arch. Derm. Syph., N.Y.*, XXXI., 291.

KELLOGG, F., EPSTEIN, N. N., and KERR, W. J., 1936, Hepatic complications in the treatment of syphilis. II. Incidence of hepatic disease in patients with untreated syphilis and during their subsequent treatment. *Ann. intern. Med.*, LVII., 666.

KRAFT, R. M., HARRIS, S., Jr., ROBINSON, C. S., and GILLIAND, H., 1938, Quantitative studies on arsenic distribution and excretion after intravenous injections of neoarsphenamine. *Amer. J. Syph.*, XXII., 215.

LAMB, C. A., and BLAKELY, E., 1937, A study of phosphatase elevation in neoarsphenamine administration. *New. Eng. J. Med.*, CCXVII., 353.

LAMBERT, L., 1937, De l'emploi d'une solution de glycocolle comme solvant des arsénobenzènes dans le traitement de la syphilis chez les indigènes du Sénégal.

LANDSTEINER, K., and JACOBS, J., 1935, Studies on the sensitization of animals with simple chemical compounds. *J. exp. Med.*, LXI., 643.

LANDSTEINER, K., and JACOBS, J., 1936, Studies on the sensitization of animals with simple chemical compounds. III. Anaphylaxis induced by arsphenamine. *Ibid.*, LXIV., 717.

LAUF, R., 1935, Untersuchungen über die Salvarsanüberempfindlichkeit des Meerschweinchens. *Z. ImmunForsch.*, LXXXVI., 49.

LERMAN, J., 1929, A comparison of arsphenamine and catarrhal jaundice with special reference to the blood picture. *Amer. J. med. Sci.*, CLXXVIII., 54.

LEWIN, E. M., 1933, Neues zur Lehre von der toxischen Wirkung von Arsenobenzolpräparaten auf die Leber. *Arch. Derm. Syph., Wien*, CLXVII. 487.

McLACHLAN, A. E. W., 1936, Calcium thiosulphate in treatment of complications of arsphenamine therapy in syphilis. *J. Chemother.*, XIII., 127.

MADERNA, C., 1934, Le epatoamine nella intolleranza da arsenobenzolo. *Rif. med.*, L., 640.

MASSON, L. V., 1925, Pathogénie clinique et expérimentale des ictères dits arsénobenzoliques. *Thèse de Strasbourg.*

MATTICE, M. R., and WEISMAN, D., 1937, Urinary excretion of arsenic. II. The influence of thiosulphate. *Amer. J. med. Sci.*, CXCIII., 420.

MEDICAL RESEARCH COUNCIL, 1922, Reports of the Salvarsan Committee. II. Toxic effects following the employment of arsenobenzol preparations. *Special Rept., No.* 66. London: H.M. Stationery Office.

Milian, G., 1920, Ictère et arsenobenzol. *Médecin, Paris*, II., 113.

Milian, G., 1934, Accidents cutanés des médicaments antisyphilitiques. *Ann. Mal. vénér.*, XXIX., 553.

Murray, D. H., 1930, Acriflavine : its use by intravenous injection in the treatment of gonorrhœa. *J. R. Army med. Corps.*, LIV., 19.

O'Leary, P., Green, T. H., and Rowntree, L., 1929, Diseases of the liver. *Arch. intern. Med.*, XLIV., 155.

Orr, H., 1933, Medical shock following the use of neoarsphenamine. *Brit. J. Dermat.*, XLV., 58.

Oser, B. L., and Sulzberger, M. B., 1934, Influence of ascorbic acid of diet on sensitization of guinea-pigs to neoarsphenamine. *Proc. Soc. exp. Biol., N.Y.*, XXXII., 716.

Phelps, J. R., 1929, Reactions incidental to the administration of 191,778 doses of neoarsphenamine and other arsenical compounds in the United States Navy. *U.S. Naval Med. Bull.*, XXVII., 205.

Rajam, R. V., 1935, Some of the major complications in treatment of syphilis. *Indian med. Gaz.*, LXX., 661.

Ravaut, P., 1920, L'importance des traitements internes en dermatologie. L'emploi du cacodylate de soude à hautes doses et de l'hyposulfite de soude. *Pr. méd.*, XXVIII., 73.

Rebaudi, U., 1934, Le foie à l'état d'acide aminé, consideré comme un moyen préventif des accidents de l'arsénobenzénothérapie. Réalisation d'un traitement massif simple et bien toleré de la syphilis. *Ann. Mal. vénér.*, XXIX., 481.

Richards, H. R. M., 1933, Some aspects of syphilis therapy. *Trans. Derby med. Soc.* (circulated privately).

Robinson, H. M., 1934, Patch tests in the determination of arsphenamine sensitization. *Sth. med. J.*, XXVII., 845.

Robinson, H. M., 1936, Intravenous testing in postarsphenamine dermatitis. *Ibid.*, XXIX., 411.

Ruge, H., 1932, Die Zusammenhänge zwischen Syphilis, Salvarsan und der sogenannten katarrhalischen Gelbsucht auf Grund von 2,250 in der Marine von 1919–1929 beobachteten Fällen. *Derm. Wschr.*, XCIX., 278.

Russell, D. S., 1937, Changes in the central nervous system following arsphenamine medication. *J. Path. Bact.*, XLV., 357.

Sager, R. V., 1936, Factors responsible for jaundice in syphilis with special reference to the *rôle* of the arsphenamines. *Arch. intern. Med.*, LVII., 666.

Schreiner, K., and Ensbruner, G., 1934, Weitere Beiträge zur Frage der allergischen Salvarsanhauterscheinungen. *Arch. Derm. Syph., Wien*, CLXX., 61.

Shaffer, L. W., 1934, Treatment of postarsphenamine dermatitis. *Arch. Derm. Syph., N.Y.*, XXIX., 173.

Shivers, G., 1933, Pulmonary embolisms following intravenous arsenical injections. Attempts to find a preventive method. *Ibid.*, XXVII., 902.

Shivers, G., 1934, Avoidance of pulmonary complications from intravenous arsenicals. *Colo. med. J.*, XXXI., 11.

Silbergleit, H., and Föckler, 1919, Ueber das Auftreten von Ikterus und akuter gelber Leberatrophie bei Syphilitikern im Anschluss an Neosalvarsanbehandlung. *Z. klin. Med.*, LXXXVIII., 333.

Slosse, M., 1921, Etude sur l'intoxication professionnelle par les arsénobenzols. *Bull. Acad. Méd. Belg.*, I., 416.

Snodgrass, W. R., and Peters, R. J., 1937, An analysis of the results of treatment of early, latent and mucocutaneous tertiary syphilis. *Medical Research Council, Spec. Rep. Ser. No.* 224. London : H.M. Stationery Office.

SOFFER, L. J., 1936, Blood electrolytic studies in experimental liver injury. *Proc. Soc. exp. Biol., N.Y.*, **XXXV.**, 160.

SOFFER, L. J., 1937, Postarsphenamine jaundice. *Amer. J. Syph.*, **XXI.** 241.

SPILLMAN, A., and KRUMHOLZ, B., 1936, Résultats de l'emploi du glycocolle en solution à 4 pour cent comme méthode de prophylaxie des accidents dus aux novarsénobenzènes. (Méthode de Jean Benech.) *Bull. Soc. franç. Derm. Syph.*, **XLIII.**, 203.

STEPHENSON, C. S., and HARRISON, W. T., 1938, The effect of the age of neoarsphenamine on reaction expectancy. *Pub. Hlth. Bull.*, **LIII.**, 945.

STOKES, J. H., and CATHCART, E. P., 1923, Contributing factors in post-arsphenamine dermatitis with special reference to the influence of focal and intercurrent infections. *Arch. Derm. Syph., N.Y.*, **VII.**, 14.

STOKES, J. H., and KULCHAR, G. V., 1934, The infection-allergic complex in arsphenamine dermatitic reactions with special reference to dermataphytosis. *Brit. J. Derm.*, **XLVI.**, 134.

STOKES, J. H., RUEDEMANN, R., and LEMON, W. S., 1920, Epidemic infectious jaundice and its relation to the therapy of syphilis. *Arch. intern. Med.*, **XXVI.**, 521.

SWIFT, H. F., 1912, Anaphylaxis to salvarsan. *J. Amer. med. Ass.*, **LIX.**, 1236.

VILLARET, M., JUSTIN-BESANÇON, L., and DESOILLE, H., 1937, L'opothérapie hépatique au cours des chimothérapies antisyphilitiques. *Nutrition, Paris*, **III.**, 143.

WEINBERG, T., 1937, Medical shock following intravenous therapy with neoarsphenamine. *Amer. J. Syph.*, **XXI.**, 376.

WERNER, S., 1897, Beiträge zur Pathologie des Ikterus syphiliticus. *Münch. med. Wschr.*, **XLIV.**, 736.

WILCOX, W., 1933, Toxic jaundice and antisyphilitic treatment. *Brit. J. vener. Dis.*, **IX.** 65.

WILE, U. J., and SAMS, W. M., 1934, A study of jaundice in syphilis. *Amer. J. med. Sci.*, **CLXXXVII.**, 297.

NEW ARSENIC DERIVATIVES

The ideal chemotherapeutic agent for the treatment of syphilis, as for other infections, should fulfil certain conditions. The drug should be easily prepared and of constant composition. It should be cheap and readily available, simple in form and easily administered. It should produce a minimum of untoward reactions, either immediate or delayed, and finally it should be rapid in action.

It is obvious that, despite their undoubted value, the anti-syphilitic compounds so far thoroughly investigated, do not comply with the above conditions. A number of other arsenical drugs has therefore been prepared. In animals certain of these drugs appear to possess satisfactory chemotherapeutic indices, but the majority have not been extensively studied in the treatment of human syphilis, and until such studies have been carried out it is not possible to assess their value.

The arsenic derivatives may be divided into tervalent compounds, which have little effect in neurosyphilis, and quinquevalent compounds, which are of special value in syphilis of the nervous system.

TERVALENT COMPOUNDS

(i.) SOLU-SALVARSAN

In order to overcome the inconvenience of having to dissolve the arsphenamines immediately before use, a soluble arsphenamine preparation has been placed on the market by the I.G. Farben Industrie. The solution, 1 c.c. of which contains 0·02 gm. of tervalent arsenic, is slightly alkaline, keeps well at ordinary temperatures, and can be injected either intramuscularly or intravenously. The toxicity for mice of 20 gm. weight is 0·3 to 0·5 c.c., while 0·25 c.c. is well tolerated. For rats the toxic dose is 5 to 7 c.c. per kilogram of body weight, the tolerated dose being 4 c.c.; in rabbits the toxic dose is 3 c.c. and the tolerated dose 0·5 c.c. per kilogram of body weight. According to Rothermundt and Richter (1935) the curative dose in rabbit syphilis is 0·15 c.c. per kilogram of body weight. The compound has been successfully used in human syphilis by van Putte (1935), Riehl (1935) and others.

References

RIEHL, G., Jr., 1935, Über die intravenöse Anwendung des Solusalvarsan. *Wien. med. Wschr.*, LXXXV., 1052.

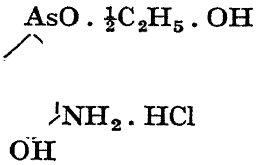
ROTHERMUNDT, M., and RICHTER, R., 1935, Experimentelles ueber Solusalvarsan. *Z. ImmunForsch.*, LXXXV., 474.

VAN PUTTE, P. J., 1935, Neosalvarsaan, Myosalvarsaan or Solusalvarsaan? *Ned. Tijdschr. Geneesk.*, LXXIX., 3031.

(ii.) MAPHARSEN

Among the many arsenical preparations originally investigated by Ehrlich and Hata (1910) was 3-amino-4-hydroxyphenylarsenious oxide. As, however, the toxicity of this compound was thought to be greater and its therapeutic index less than that of arsphenamine, it received little attention, more especially since Voegtlin and his colleagues (1923) came to the conclusion that the increased toxicity of the arsphenamines on standing was due to the formation of 3-amino-4-hydroxyphenylarsenious oxide, while Rosenthal and Probey (1933) had shown that the toxicity of 3-amino-4-hydroxyphenylarsenious oxide is nearly ten times that of arsphenamine. Interest was reawakened, however, in this compound by the work of Tatum and Cooper (1934), who found that in rabbit syphilis its chemotherapeutic index was 1 : 1·66 as compared with 1 : 1·4 for neoarsphenamine, while in experimental trypanosomiasis the indices were 1 : 25 for 3-amino-4-hydroxyphenylarsenious oxide, and from 1 : 20 to 1 : 40 for various brands of neoarsphenamine. Gruhzit (1934 and 1935) obtained an index of 1 : 9 for infections with *Trypanosoma equiperdum* in rats as compared with 1 : 11·6 for arsphenamine and 1 : 8·3 for neoarsphenamine. The chemotherapeutic index in rabbit syphilis was 1 : 4 for 3-amino-4-hydroxyphenylarsenious oxide and 1 : 3·75 for neoarsphenamine, given in single doses : in repeated doses the indices were 1 : 4·6 and 1 : 1·67. These results have not been entirely confirmed, for Raiziss and Severac (1935) found that in rabbit syphilis the indices were 1 : 0·95 for 3-amino-4-hydroxyphenylarsenious oxide and 1 : 5 for neoarsphenamine, and in experimental trypanosomiasis 1 : 7·2 and 1 : 20 respectively, neoarsphenamine being thus from three to five times as efficient.

Notwithstanding these conflicting results, the hemialcoholate of 3-amino-4-hydroxyphenylarsenious oxide hydrochloride has now been used in the treatment of a number of cases of human syphilis under the name of "Mapharsen," "Mapharside," or "Arsenoxide."



$AsO \cdot \frac{1}{2}C_2H_5 \cdot OH$; $NH_2 \cdot HCl$; OH

Mapharsen

In experimental animals Gruhzit (1934) found that doses of 1 to 2 mgm. per kilogram of body weight cause no pathological lesions though changes are produced when the dose is raised to 4 or 5 mgm. per kilogram of body weight. The maximum tolerated dose for rats injected intravenously is 18 mgm. per kilogram of body weight (Gruhzit, 1935), the minimum lethal dose, 20 mgm. per kilogram of body weight: injected intramuscularly the maximum tolerated dose is 22 mgm. and the minimum lethal dose 24 mgm. per kilogram of body weight. In rabbits, the maximum tolerated dose given intravenously is 14 mgm., and the minimum lethal dose 16 mgm. per kilogram of body weight.

In experimental rabbit syphilis 1 mgm. per kilogram of body weight of mapharsen causes as rapid a disappearance of spirochætes and as rapid healing as 10 mgm. of neoarsphenamine, while a complete cure is obtained in 80 per cent. of rabbits by the same dose.

Mapharsen contains approximately 29·01 per cent. of tervalent arsenic, and is a white amorphous powder, readily soluble in water. Aqueous solutions are acid to methyl red, but alkaline to congo red. When exposed to air or moisture mapharsen gradually oxidises to a less toxic phenylarsonic acid. It is freely soluble in alcohols, acids, alkalis and alkali carbonates, and is not precipitated by tissue fluids.

Mapharsen is excreted in the urine and fæces in the ratio of 1 : 1·65. From 85 to 96 per cent. of arsenic is excreted in eleven days.

The first experiments on the treatment of human syphilitics were made by Foerster and his colleagues (1935), who reported disappearance of spirochætes from primary lesions after one injection and rapid healing. Similar results have now been obtained by Kulchar and Barnett (1936), Gruhzit, Dixon *et al.* (1936), Astrachan (1937), Parsons (1937), Wieder, Foerster and Foerster (1937), Schmidt and Taylor (1937), Peterson (1937) and Marshall (1937). From these combined observations on patients it is possible to deduce the following results : Mapharsen is of little or no use in neurosyphilis, but in primary and early secondary syphilis it brings about rapid healing of lesions with disappearance of spirochætes in twenty-four hours. In early cases the Wassermann reaction of the blood is changed to negative, though Kulchar and Barnett (1936) found that the rate of reversal was somewhat slower than with the arsphenamines. Returns to a positive reaction may occur, as with clinical relapses, more especially when the periods of therapy have been short or irregular. After continuous treatment for a year Gruhzit and his colleagues observed no patients with pathological spinal fluids, though when treatment which should have been compressed into fifty-two weeks was spread over from sixty-five to eighty-four weeks, 3 of 11 patients had pathological fluids. Kulchar and Barnett (1936), after at least six months of regular treatment, found that of 21 patients the cerebro-spinal fluids were normal in 15, slightly pathological in 2, moderately pathological in 3, and paretic in type in 1.

Mapharsen should be given in initial doses of 40 mgm. per kilogram of body weight for a woman, and 60 mgm. for a man, one injection being given intravenously for eight to ten weeks, alternating with courses of either bismuth or mercury. The maximum weekly dose should be regarded as 0·06 gm. for children : 0·5 mgm. per kilogram of body weight is usually satisfactory.

The safe dose of mapharsen is thus much less than that of the arsphenamines. In contra-distinction to the arsphenamines, intravenous injections should be given rapidly, otherwise pain in the arm is common. Schmidt and Taylor (1937) recommend twice weekly injections during the first course, then once a week, 8 to 12 injections constituting a course.

Immediate reactions, such as nitritoid crises, are extremely rare, while those involving the skin and liver are uncommon. Foerster *et al.* (1935) reported severe Herxheimer reactions, jaundice in 4 out of 80 patients, and accentuation of renal damage in 4 other patients. Schmidt and Taylor (1937), on the other hand, had no case of hepatitis or exfoliative dermatitis among 1,400 patients. Two types of reaction are, however, common : (1) mild erythema which disappears quickly and does not prevent further medication ; and (2) nausea which disappears on giving Lugol's solution by mouth. Both Falconer, Epstein and Wever (1936) and Marshall (1937) emphasize the fact that in patients who are extremely sensitive to the arsphenamines, mapharsen can be given with impunity. Jordan and Traenkle (1937) found that of 64 patients who had had repeated severe gastro-intestinal reactions when treated with the arsphenamines, 53 tolerated mapharsen well : 20 patients who had had severe nitritoid crises after administration of arsphenamines all tolerated mapharsen perfectly, while of 18 patients with post-arsphenamine jaundice, 16 showed no reaction to mapharsen.

The advantages which are claimed for mapharsen over the arsphenamines are :—

(1) The greater therapeutic effect with smaller doses of arsenic.

(2) Less local discomfort if the solution is accidentally extravasated into the tissues.

(3) Less disagreeable taste.

(4) Greater toleration.

(5) Absence of nitritoid crises and severe skin reactions.

(6) Decreased toxicity to the liver.

Further observations are necessary to determine whether these apparent advantages are maintained.

References

ASTRACHAN, G. D., 1937, Mapharsen in antisyphilitic therapy: preliminary report: study based on 3,386 injections of mapharsen. *Amer. J. Syph.*, XXI., 81.

EHRLICH, P., and HATA, S., 1910, "Die experimentelle Chemotherapie der Spirillosen." Berlin: Julius Springer.

FALCONER, E. H., EPSTEIN, N. N., and WEVER, G. K., 1936, Purpura hæmorrhagica following the administration of neoarsphenamine. The reaction to neoarsphenamine compared with the reaction to mapharsen. *Arch. intern. Med.*, LVIII., 495.

FOERSTER, O. H., MCINTOSH, R. L., WIEDER, L. M., FOERSTER H. R. and COOPER, G. A., 1935, Mapharsen in the treatment of syphilis. *Arch. Derm. Syph., N.Y.*, XXXII., 859.

GRUHZIT, O. M., 1934, Arsenoxide in experimental animals. *Arch. Path.*, XVIII., 582.

GRUHZIT, O. M., 1935, Mapharsen ("Arsenoxide") in the therapy of experimental syphilis and trypanosomiasis. *Arch. Derm. Syph., N.Y.*, XXXII., 848.

GRUHZIT, O. M., DIXON, R. S., MCALONAN, W. T., SHERMAN, W. L., MERRIMAN, K. S., SEWELL, G., ARNOLD, E., DOWLING, P. C., PIERSON, M., CHAMBERS, E. R., and LAUPPE, F., 1936, Mapharsen in mass treatment of syphilis in a clinic for venereal diseases. *Ibid.*, XXXIV., 432.

JORDAN, J. W., and TRAENKLE, H. L., 1937, Reactions to mapharsen with especial reference to its use in patients who react to arsphenamines. *Ibid.*, XXXVI., 1158.

KULCHAR, G. V., and BARNETT, C. W., 1936, Mapharsen in the treatment of early syphilis. *Amer. J. Syph.*, XX., 482.

MARSHALL, J. W., 1937, The treatment of syphilis with mapharsen. *Ibid.*, XXI., 645.

PARSONS, R. P., 1937, Estimate of arsenoxide (mapharsen) in the treatment of early syphilis. *Nav. med. Bull., Wash.*, XXXV., 207.

PETERSON, S. C., 1937, The use of mapharsen in the treatment of syphilis. *Canad. med. Ass. J.*, XXXVI., 172.

RAIZISS, G. W., and SEVERAC, M., 1935, Comparative chemotherapeutic studies of "arsenoxide" (3-amino-4-hydroxyphenylarsenoxide) and neoarsphenamine. *Amer. J. Syph.*, XIX., 473.

ROSENTHAL, S. M., and PROBEY, T. F., 1933, The arsenoxide content and toxicity of fresh and old samples of arsphenamine. *J. Pharmacol.*, XLI., 286.

SCHMIDT, L. E., and TAYLOR, G. G., 1937. The treatment of syphilis with mapharsen. *Amer. J. Syph.*, XXI., 402.

TATUM, A. L., and COOPER, G. A., 1934, An experimental study of mapharsen (meta-amino-parahydroxyphenylarsine oxide) as an antisyphilitic agent. *J. Pharmacol*, L., 198.

VOEGTLIN, C., DYER, H. A., and LEONARD, C. S., 1923, On the mechanism of the action of arsenic upon protoplasm. *Publ. Hlth. Rep., Wash.*, XXXVIII., 1882.

WIEDER, L. M., FOERSTER, O. H., and FOERSTER, H. R., 1937, Mapharsen in the treatment of syphilis. Further experiences. *Arch. Derm. Syph., N.Y.*, XXXV., 402.

(iii.) TRISODARSEN

The trisodium salt of 3 : 3′-diamino-4 : 4′-dihydroxyarsenobenzene-N : N′-dimethylenesulphonic acid, known as "trisodarsen" or "triarsen," was synthesized by Raiziss and has been studied by Stokes and Beerman (1937). Trisodarsen, which is thus merely a variant of sulpharsphenamine, is a water-soluble yellow powder, containing not less than 19 per cent. of tervalent arsenic. It is more stable in air than either neoarsphenamine or sulpharsphenamine. Rats are said to tolerate doses of from 500 to 600 mgm. per kilogram of body weight, given intravenously, while the maximum tolerated dose for neoarsphenamine is from 240 to 400 mgm. per kilogram of body weight. Single doses of 25 mgm. of trisodarsen per kilogram of body weight are said to cure experimental syphilis in the rabbit, in which species the chemotherapeutic index is 1 : 18, neoarsphenamine in similar circumstances being from 1 : 6 to 1 : 10. Trisodarsen was given intravenously to 189 patients, almost all with early syphilis, the initial dose being 0·3 gm., increased to 0·5 to 0·6 gm. Since spirochætes disappeared from the initial lesions in from twenty-four to ninety-six hours, trisodarsen equals the arsphenamines and mapharsen in this respect. In the healing of primary and secondary lesions trisodarsen is said to be superior to neoarsphenamine and silver arsphenamine, but rather slower than mapharsen and definitely slower than arsphenamine. In the case of primary syphilitics the time in days for reversal to negative was rather longer than with arsphenamine, but shorter than with neoarsphenamine. In secondary syphilis the time for reversal was shorter than with arsphenamine and much shorter than with neoarsphenamine. In 78 per cent. of patients serological reactions had been reversed by the tenth injection. After six months or more, 8·9 per cent. of relapses had occurred. Only 4·3 per cent. of patients had abnormal spinal fluids.

Although Herxheimer effects were rare and there were no injuries affecting the cardiovascular, hæmatopoietic or nervous systems, there were possibly more instances of dermatitis than in a similar series of cases treated with neoarsphenamine; of 165 patients, 5 developed dermatitis, in 2 cases very severe. With trisodarsen, cutaneous reactions occurred once in 1,338 injections, as against once in 5,367 injections in the case of neoarsphenamine. Nitritoid crises occurred in 13, or 8 per cent., of patients, while of 165 patients no fewer than 33 had symptoms of nausea and vomiting : in 12 these symptoms were so severe as to stop further administration. Despite the frequency of reactions involving the skin and alimentary system, trisodarsen appears to be of value in early syphilis, but further tests are required on the effect of this compound on later cases of syphilis.

Reference

STOKES, J. H., and BEERMAN, H., 1937, New arsphenamine synthetics in treatment of syphilis. A consideration of test procedure and of a new drug (triarsen). *Arch. Derm. Syph., N.Y.*, **XXXV.**, 78.

THE ACTION OF TRISODARSEN COMPARED WITH ARSPHENAMINE AND NEOARSPHENAMINE

Average Time and Treatment necessary for Disappearance of Visible Lesions in Man

Drug	No. of cases	Average total dosage in gm.	Average number of injections	Average number of days
Arsphenamine . . .	32	1·3	2·7	14
Neoarsphenamine . .	61	3·8	5·8	23
Trisodarsen . . .	53	1·35	2·7	14·6
Trisodarsen and bismuth .	62	1·05	2·66	15·0

Average Time and Treatment necessary for Reversal of Blood Wassermann Reaction to Negative

Drug	No. of cases	Average total dosage in gm.	Average number of injections	Average number of days
Arsphenamine . . .	103	3·9	9·7	75
Neoarsphenamine . .	110	7·1	13·4	99
Trisodarsen . . .	40	4·18	8·0	58·7

(iv.) THIOARSENE

The possibility that arylthioarsinites, formed by the interaction of a thiol acid with an arylarsenious oxide, may be of value in syphilis was investigated by Eckler and Shonle (1935), who found that of twenty compounds, one, a disodium bis-(parasulphophenyl) (acetamidophenyl)-diarylthioarsinite, was possessed of trypanocidal properties in experimentally infected rats and spirochæticidal properties in experimental rabbit syphilis in doses of 20·6 mgm. per kilogram of body weight. Clinical observations were carried out by Becker and Obermayer (1935), Connor and his colleagues (1935) and Robinson and Moore (1935). The last-named authors, however, reported a number of severe gastro-intestinal reactions and believed that this compound is of little value in the treatment of syphilis.

References

Becker, S. W., and Obermayer, M. E., 1935, Clinical observations on the treatment of syphilis by a combination of bismuth salicylate and a new arsenical synthetic. A preliminary report. *Amer. J. Syph.*, XIX., 505.

Connor, W. H., Shaw, H. C., Levin, E. A., and Palmer, R. B., 1935, The therapeutic value of thio-arsene: a clinical study of the therapeutic efficiency and toxicity of disodium bis-(*p*-sulfophenyl)(acetamidophenyl)-dithioarsenite, based on 2,282 injections administered to 251 patients with syphilis. *Ibid.*, XIX., 514.

Eckler, C. R., and Shonle, H. A., 1935, A preliminary study of thio-arsene, disodium bis-(*p*-sulfophenyl)(acetamidophenyl)-dithio-arsenite. *Ibid.*, XIX., 495.

Robinson, H. M., and Moore, J. E., 1935, The treatment of syphilis with a new arsenical drug. *Ibid.*, XIX., 525.

QUINQUEVALENT DERIVATIVES

(i.) ALDARSONE

The formaldehyde sulphoxylate of 3-amino-4-hydroxyphenylarsonic acid, "Aldarsone," was prepared by Raiziss, Severac and Kremens (1934) by combining one molecule of 3-amino-4-hydroxyphenylarsonic acid with one molecule of sodium formaldehyde sulfoxylate. The arsenic and sulphur content of different samples varied somewhat, the average being 18 per cent. of arsenic and 7 per cent. of sulphur. The compound is a white crystalline powder, very soluble in water, insoluble in alcohol, ether, acetone, or chloroform. On intravenous injection the product is tolerated by rats in doses of 1·2 gm. per kilogram of body weight, by rabbits in doses of 0·4 gm, per kilogram of body weight. Upon oral administration to rabbits the maximum tolerated dose was 0·4 gm. In rabbits infected with syphilis a single dose of either 0·1 or 0·2 gm. per kilogram of body weight is sufficient to cause spirochætes to disappear in from three to seven days. The minimum sterilizing dose when given by mouth, in ten weekly treatments, was between 0·05 and 0·10 gm. per kilogram of body weight. Prophylactic experiments on rabbits inoculated intraspinally with spirochætes indicated a fair degree of penetration of the drug into the central nervous system.

Aldarsone has been used in the treatment of trichomonas infection of the vagina by First and Rakoff (1936), but does not appear to have been tested in human syphilis.

References

First, A., and Rakoff, A. E., 1936, Aldarsone in the treatment of a resistant case of trichomonas vaginitis. Clinical and laboratory observations of a case treated by a variety of methods. *J. Chemother.*, XIII., 54.

Raiziss, G. W., Severac, M., and Kremens, A., 1934, Experimental chemotherapy of preparation No. 1717, formaldehyde sulfoxylate of 3-amino-4-hydroxyphenylarsonic acid. *Ibid.*, XI., 34.

(ii.) THE SODIUM SALT OF 3-AMINO-4-β-HYDROXY-ETHOXYPHENYLARSONIC ACID

This compound, known as preparation "190," was first prepared by Sweet and Hamilton (1934), and has been studied experimentally in animals and clinically in man by Tatum and his colleagues (1937).

Although in rabbit syphilis the sterilization of lymph nodes and reversal of the Wassermann reaction occurs more rapidly with "190" than with tryparsamide, the action is not as rapid as with some of the tervalent arsenicals. The rate of excretion is the same as that of tryparsamide, as are the toxic manifestations when large doses are given to animals. In the treatment of human syphilis doses of either 1 or 3 gm. were given subcutaneously, the former dose twice, the latter once a week. The injections were given for from eight to twenty-five weeks, followed by rest periods of from six to eight weeks, mercury salicylate being given intramuscularly after the first four months. Of 80 cases 15 showed considerable clinical improvement, 35 some improvement, and 32 no improvement. Although serological improvement was more rapid than in the case of tryparsamide, cases of toxic amblyopia were more frequent: two patients became completely and one almost blind. This tendency to involve the optic nerves renders doubtful the value of this compound.

References

Sweet, L. A., and Hamilton, C. S., 1934, Arsenated phenoxyalkanols. *J. Amer. chem. Soc.*, LVI., 2409.

Tatum, A. L., Pfeiffer, C. C., Kuhs, M. L., Lorenz, W. F., and Green, J. T., Jr., 1937, A study of the sodium salt of 3-amino-4-β-hydroxethoxyphenylarsonic acid in experimental trypanosomiasis and syphilis and in clinical syphilis and neurosyphilis. *J. Pharmacol.*, LIX., 241.

(iii.) NEOCRYL

Sodium succinanilomethylamide-*p*-arsonate, *neocryl*, was studied by Yorke, Murgatroyd, Glyn-Hughes, Lester and Ross (1936) and found to exert an action not only in trypanosomiasis, but in primary, secondary and tertiary syphilis. The trypanocidal action of this compound has already been discussed (*cf.* p. 160). In man weekly intravenous doses of from 2 to 4 gm. are well tolerated when given as a 15 to 20 per cent. solution in sterile distilled water. In primary and secondary syphilis healing of the lesions occurred, but of 4 patients, 2 relapsed. In tertiary syphilis 20 out of 25 patients showed considerable improvement, but in 2 the lesions subsequently broke down again. The result of treating early neurosyphilis and taboparesis was also promising, since improvement occurred in 5 out of 8 patients. Patients who were sensitive to the arsphenamines showed no sensitivity to neocryl. Apart from occasional nausea, vomiting and, very rarely, mild dermatitis and jaundice, no reactions occurred, visual disturbances being entirely absent.

Reference

YORKE, W., MURGATROYD, F., GLYN-HUGHES, F., LESTER, H. M. O., and ROSS, A. O. F., 1936, Neocryl, a new arsenical for the treatment of syphilis and trypanosomiasis. *Brit. Med. J.*, *i.*, 1042.

(iv.) ARSENICAL DERIVATIVES OF PYRIDINE

A number of derivatives of pyridine containing arsenic prepared by Binz (1931) and Binz and Maier-Bode (1933 and 1936) have been tested for their trypanocidal action by Schlossberger and Schüffner (1934 [1 & 2]) and for antisyphilitic activity by Schlossberger (1937) in rabbits and mice: the toxicity of a number of these compounds for mice has been studied by Christison (1934 [1 & 2]). The compounds which showed some curative action in mice and rabbits infected with syphilis are shown in the table.

THERAPEUTIC ACTION OF ARSENICAL DERIVATIVES OF PYRIDINE IN EXPERIMENTAL MOUSE AND RABBIT SYPHILIS (Schlossberger, 1937) *

PREPARATION		ACTION IN MOUSE SYPHILIS								
No.	Compound	Interval in weeks between infection and treatment	Dose in gm. per 20 gm.	Route of inocula-tion.	Result of inoculating tissues into rabbits	Interval in days between infection and treatment	Dose in gm. per kilogm.	Route of inocula-tion	Action on primary lesion and spirochætes	Result of inoculating lymph nodes in fresh rabbits
BR 1	2-Pyridone-5-arsonic acid. M.	1 4	0·081 0·10	sc. sc.	— —	41	0·75	intraven.	Rapid healing: spirochætes —ve after 24 hours.	+
BR 23	3-Amino-2-pyridone-5-arsonic acid. M.	1 5 13	0·08 0·10 0·10	sc. sc. sc.	— — —	76 47	0·75 0·25	intraven.	Rapid healing: spirochætes —ve after 24 hours.	—
BR 25	2-Hydroxy-3-acetamino-pyridine-5-arsonic acid. D.	1 5 13	0·05 0·06 0·06	sc. sc. sc.	— — —	26	0·30	intraven.	Prompt healing: recurrence after 7 weeks.	
BR 34	2-Pyridone-5-arseno-1′-(3′-amino-4-hydroxy) benzene. D.	1 6 13 15	0·003 0·001 0·001 0·002	intraven. intraven. intraven. sc.	+ — — —	43	0·02	sc.	Prompt healing: spirochætes —ve after 5 days.	—
BR 45	3-Bromo-2-pyridone-5-arsonic acid. M.	1 4 5	0·04 0·05 0·08	sc. sc. sc.	— — —	43	0·25	intraven.	Not very rapid.	+
BR 47	*N*-methyl-2-pyridone-3-arsonic acid. M.	1 13	0·26 0·33	sc. sc.	+ —					
BR 68	2-Pyridone-5-arseno-5′-(2′-hydroxyphenyl-glycine). M.	10 9 15 52	0·0016 0·0016 0·002 0·002	intraven. intraven. intraven. intraven.	+ + — —					
1037	*N*-Dihydroxypropyl-2-pyridone-5-arsonic acid. M.	1 4	0·02–0·03 0·05	sc. sc.	— —	85	0·5	sc.	Moderate rate.	—
1049	2-pyridone-5-arsonic acid-*N*-acetarsanilate.	1 12	0·062 0·062	sc. sc.	— —					
1132	2-Ethoxypyridine-5-arsonic acid (dissolved in sodium carbonate solution).	1 17	0·071 0·071	intraven. intraven.	— +					
1235	2 : 3-Dihydroxy-pyridine-5-arsensulphide (in sodium carbonate solution).	3	0·001	intraven.	—					

* M = monosodium salt. D = disodium salt.

References

Binz, A., 1931, Chemistry of uro-selectan. *Brit. J. Urol.*, II., 348.

Binz, A., and Maier-Bode, H., 1933, Die Wirkung von Jodpyridonderivaten auf Streptokokken. *Biochem. Z.*, CCLVII., 351.

Binz, A., and Maier-Bode, H., 1936, Über Arsen- und Jodverbindungen der Pyridinreihe. *Z. angew. Chem.*, XLIX., 486.

Christison, M. H., 1934[1], Die Heilwirkung einer Reihe von organischen Arsenverbindungen bei der experimentellen Schweinerotlaufinfektion der weissen Maus. *Zbl. Bakt.* Abt. I. Orig., CXXXI., 193.

Christison, M. H., 1934[2], Ueber chemotherapeutische Versuche bei der Rattentrypanose. *Ibid.*, CXXXII., 228.

Schlossberger, H., 1937, Experimentelle Untersuchungen über die therapeutische Wirksamkeit von Arsenpyridinverbindungen bei Syphilis. *Med. Klinik.*, XXXIII., 1703.

Schlossberger, H., and Schüffner, R., 1934[1], Festigungsversuche an Trypanosomen mit Arsenpyridinderivaten. *Z. angew. Chem.*, XLVII., 768.

Schlossberger, H., and Schüffner, R., 1934[2], Chemotherapy of syphilis and other infectious diseases: drug-resistance experiments with arsenopyridine derivatives on trypanosomes. *J. Chemother.*, XI., 81.

THE ACTION OF TER- AND QUINQUEVALENT ARSENICALS IN SYPHILIS

In discussing the mechanism by which tervalent and quinquevalent arsenicals destroy trypanosomes both *in vitro* and *in vivo*, evidence was brought forward in favour of the view put forward by Voegtlin, Dyer and Leonard (1923), who believed that all arsenicals, whether quinque- or tervalent, are reduced or oxidised respectively to the corresponding arsenoxides by the tissues of the host.

Arsenobenzene compounds thus (Voegtlin and Smith, 1920) exert their lethal action *in vivo* both on trypanosomes and spirochætes after oxidation to the arsenoxide:

$$\mathrm{R.As = As.R + O_2 \rightarrow 2RAsO}$$

Both *in vivo* and *in vitro*, however, the action of a compound such as neoarsphenamine is more rapidly lethal to trypanosomes than to spirochætes. This difference Simić (1923) believes is due to the fact that neoarsphenamine can be absorbed by trypanosomes in the unchanged condition, whereas oxidation to the arsenoxide is necessary before it can enter the spirochætes. In this connection the greater therapeutic efficiency and higher toxicity of neoarsphenamine to the host on subcutaneous, as compared with intravenous, injection, may be partly due to the more rapid production of arsenoxide by the cells of the host. More rapid excretion is, however, also a factor.

The difference in the action of neoarsphenamine on trypanosomes and spirochætes has also been investigated by Papamarku (1927), who found that when a mixture of trypanosomes and spirochætes was treated with different concentrations of neoarsphenamine, the trypanosomes were killed in two hours by a dilution of 1 in 10 millions, while the spirochætes remained healthy for much longer in higher concentrations. Eventually, however, they were killed after twenty-four hours by a dilution of 1 in 500,000. It may therefore be concluded that in the case of trypanosomes the neoarsphenamine is rapidly taken up and becomes oxidized to the arylarsenious oxide, either on the surface or within the cell, by the normal oxidation processes of the trypanosome. In the case of the spirochætes either the rate of absorption or the rate of oxidation to the arylarsenious oxide is much slower. Eagle and Mendelsohn (1938) point out that the rate at which the antispirochætal action of the arsphenamines proceeds *in vitro* depends on numerous variables. Thus with increase in temperature from 23° to 37° C. the rate increases while serum and tissues retard the spirochæticidal effect. Arsphenamine and neoarsphenamine possess only a quarter of the spirochæticidal effect *in vitro* of arylarsenious oxide.

References

EAGLE, H., and MENDELSOHN, W., 1938, On the spirochæticidal action of the arsphenamines on *Spirochæta pallida in vitro*. *Science*, LXXXVII., 194.

PAPAMARKU, P., 1927, Versuche über die Wirkung chemotherapeutischer Stoffe . . . auf Spirochäten und Trypanosomen *in vitro*. *Z. Hyg.*, CVII., 407.

SIMIĆ, T. V., 1923, Untersuchungen über die Wirkungsweise des Neosalvarsans. *Z. Hyg.*, XCIX., 417.

VOEGTLIN, C., DYER, H. A., and LEONARD, C. S., 1923, On the mechanism of the action of arsenic upon protoplasm. *Publ. Hlth. Rep., Wash.*, XXXVIII., 1882.

VOEGTLIN, C., and SMITH, H. W., 1920, The trypanocidal action of arsenic compounds. *J. Pharm.*, XV., 475.

BISMUTH

Although in 1889 Balzar had suggested the use of bismuth in the treatment of syphilis, it was not until 1916 that Robert and Sauton demonstrated the curative action of bismuth compounds in fowl spirochætosis. These observations passed unnoticed till 1921, when Sazerac and Levaditi demonstrated the therapeutic activity of sodium potassium tartrobismuthate in experimental rabbit syphilis and in a limited number of human syphilitics. Since then a seemingly inexhaustible supply of new bismuth compounds has been produced—there are now more than 200 of them—often with little or no preliminary study to determine whether they represent any improvement on those already known. It is a moot point whether the time is not rapidly approaching when some international standardization should be adopted as in the case of the arsphenamines. Such a standardization would doubtless have been enforced long ago were it not that bismuth salts are possessed of so small a degree of toxicity.

At present the variation in actual Bi content is from 4 to 98 per cent. Such variations, however, signify little, since the amount of metallic bismuth necessary to cure experimental syphilis in the rabbit is extremely small. The chemotherapeutic activity of any compound, therefore, depends less on its metallic content than on chemical constitution, rate of absorption, rate of excretion and powers of penetration (*cf.* Forst, 1935). The fact that insoluble bismuth compounds such as bismuth salicylate are poorly, irregularly and incompletely absorbed and may become encapsulated, with subsequent breakdown and sudden bismuth poisoning, contra-indicates their use. Absorbability is not, however, everything, for, as Greenbaum (1931) points out, of two compounds with almost equal rates of absorption, one may be spirochæticidal, the other not.

Hanzlik and Spaulding (1931) believe that the electrical charge carried by the Bi ion is of considerable importance in connection with permeability, more especially in regard to the power of penetrating into the cerebrospinal fluid, into which anions penetrate more readily than cations. In sodium bismuthate and sodium iodo-bismuthite, for instance, the bismuth ion is negatively charged and penetrates into the cerebrospinal fluid, while in bismuth salicylate Hanzlik and Spaulding (1931) believe that it is positively charged and therefore does not penetrate. In compounds of the metalloids with organic acids, there is, however, considerable evidence to show that the metalloid really forms a part of the anion.

By the action of the tissues, however, the positively charged bismuth ion is slowly changed and finally becomes negatively charged (Hanzlik and Richardson, 1935). A classification of bismuth compounds on these lines is of considerable interest. The more usual classification of bismuth preparations is into (1) colloidal, (2) water insoluble, (3) water soluble, and (4) fat soluble. While there is now a tendency to concentrate on fat soluble preparations, compounds of all types continue to appear, as, for instance, the preparation by Hanzlik (1937) of a stable soluble sodium bismuthate by the addition of sodium bismuthate to a mixture of tri*iso*propanolamine, propylene glycol and water (*cf.* Hanzlik, Lehman and Richardson, 1937 and 1938 [1 & 2]).

While the bismuth preparations at present in use are always given by intramuscular injection, attempts have been made to determine how far other routes of administration can be employed. In experimental rabbit syphilis inunction of bismuth salts proved quite useless (Kolmer, 1935 [2]). Controversy still surrounds the question how far bismuth preparations given by mouth are therapeutically active. Sazerac and Levaditi (1921) were unable to protect rabbits against syphilitic infection by oral administration of sodium and potassium bismuth tartrate although the incubation period was somewhat prolonged, and in view of the results obtained by intramuscular injection interest in the route of administration lapsed. Mulzer and Serefis (1934), however, claimed to have successfully treated human syphilis by oral administration, while more recently Kolmer (1935 [1]) has reported rapid healing and apparently complete recovery in about 42 per cent. of rabbits by the oral administration for twenty-four days of 0·02 gm. of potassium bismuth tartrate per kilogram of body weight. Similar results have been obtained by Rein and Sulzberger (1936) and Kolmer, Brown and Rule (1937), but Kemp and Rosahn (1936) have failed entirely to treat syphilis in the rabbit with bismuth given by mouth.

While the hopes which were at one period entertained in certain countries that bismuth could entirely replace the arsphenamines in the therapy of syphilis have been falsified, bismuth has now almost entirely replaced mercury. There is, however, according to Cannon (1937), little to choose between bismuth and mercury when used in conjunction with an arsenical preparation (*cf.* Cannon and Robertson, 1936). More than twice as many patients, however, became serologically resistant with mercury as with bismuth, but more relapses occurred with bismuth.

In the treatment of forms of endemic syphilis such as bejel, described by Hudson and Crosley (1936) in the bedouin of Syria, where owing to the character of the population the aim is control of contagion in the community rather than cure of the individual, bismuth preparations are superior to the arsphenamines. Similarly in the treatment of yaws the greater cheapness and ease of administration of bismuth render it the more suitable preparation. Saunders (1937) in Jamaica, for instance, has shown that in a treatment period of eight weeks as many as 5,000 yaws patients can be successfully treated with bismuth salicylate.

TOXIC REACTIONS

Although reactions are much less common with bismuth than with the arsphenamines, toxic sequelæ, either of a localized or general character, have been recorded.

Among the local reactions the two most important are abscess at the site of injection and embolism, associated with livid dermatitis. The generalized reactions include :—

(1) nitritoid crises, usually slight in character
(2) shock, following especially intravenous injection
(3) nephritis often with complete anuria
(4) stomatitis
(5) progressive wasting and dyspepsia

(6) jaundice
(7) skin eruptions
  (*a*) urticaria
  (*b*) papulo-squamous erythema
  (*c*) exfoliative dermatitis
  (*d*) generalized discoloration, resembling argyria (*cf.* Gougerot and Blum, 1935).

The following are rare :—

(8) polyneuritis
(9) zoster
(10) headache and fever
(11) agranulocytosis
(12) multiple hæmorrhages
(13) changes in the growing skeleton shown by X-rays analogous to the lead lines in lead poisoning (*cf.* Caffey, 1937).

Sodium thiosulphate has no effect as an antidote in bismuth poisoning, and where there is impairment of renal function the most satisfactory measure appears to be the intravenous injection of saline and glucose.

References

BALZAR, F., 1889, Expériences sur la toxicité du bismuth. *C. R. Soc. Biol.*, XLI., 537.

CAFFEY, J., 1937, Changes in growing skeleton after administration of bismuth. *Amer. J. Dis. Child.*, LIII., 56.

CANNON, A. B., 1937, Optimal treatment for early syphilis, based on a twenty-year trial of arsphenamine, bismuth and mercury preparations. *Amer. J. Syph.*, XXI., 155.

CANNON, A. B., and ROBERTSON, J., 1936, The choice of bismuth or mercury with arsphenamine. *J. Amer. med. Ass.*, CVI., 2133.

FORST, A. W., 1935, Wismut. *Handb. exp. Pharmakol.*, III., 2249. Edited by A. Heffter and W. Heubner, Berlin.

GOUGEROT, H., and BLUM, P., 1935, Imprégnation bismuthique cutanée générale. Pseudoargyrie cutanée provoquée par le bismuth. *Bull. Soc. franç. Derm. Syph.*, XLII., 276.

GREENBAUM, S. S., 1931, Concerning the curative values of certain bismuth compounds : experimental study. *Amer. J. Syph.*, XV., 59.

HANZLIK, P. J., 1937, Sodium bismuthate soluble in experimental syphilis. *J. Pharmacol.*, LIX., 328.

HANZLIK, P. J., LEHMAN, A. J., and RICHARDSON, A. P., 1937, Sodium bismuthate soluble : a new product for intramuscular and oral administration in the treatment of syphilis : a preliminary summary report. *Amer. J. Syph.*, XXI., 1.

HANZLIK, P. J., LEHMAN, A. J., and RICHARDSON, A. P., 1938[1], Sobismol : toxicity, tolerance and irritation according to different channels of administration. *J. Pharmacol.*, LXII., 372.

HANZLIK, P. J., LEHMAN, A. J., and RICHARDSON, A. P., 1938 [2], Excretion of bismuth after intramuscular injection of sobismol: experimental and clinical results. *Ibid.*, **LXII.**, 404.

HANZLIK, P. J., and RICHARDSON, A. P., 1935, Bismuth in body fluids and tissues. *J. Pharmacol.*, **LV.**, 447.

HANZLIK, P. J., and SPAULDING, J. B., 1931, Ionic migration of bismuth in different bismuth products under different conditions. *Proc. Soc. exp. Biol., N.Y.*, **XXVIII.**, 847.

HUDSON, E. H., and CROSLEY, S. S., 1936, Bismuth in the treatment of endemic syphilis (bejel). *J. trop. Med.*, **XXXIX.**, 245.

KEMP, J. E., and ROSAHN, P. D., 1936, The oral administration of bismuth in the treatment of experimental rabbit syphilis. *Amer. J. Syph.*, **XX.**, 131.

KOLMER, J. A., 1935 [1], Oral administration of potassium bismuth tartrate in treatment of syphilis. *Arch. Derm. Syph., N.Y.*, **XXXI.**, 9.

KOLMER, J. A., 1935 [2], Inunctions of bismuth in the treatment of experimental syphilis of rabbits. *J. Chemother.*, **XII.**, 278.

KOLMER, J. A., BROWN, H., and RULE, A. M., 1937, The oral administration of potassium bismuth tartrate in the treatment of experimental syphilis of rabbits with a note on the gastric chemistry of rabbits. *Amer. J. Syph.*, **XXI.**, 387.

MULZER, P., and SEREFIS, S., 1934, Die perorale Wismutbehandlung der Syphilis. *Münch. med. Wschr.*, **LXXXI.**, 1525.

REIN, C. R., and SULZBERGER, M. B., 1936, Bismuth by mouth in the treatment of syphilis. *Amer. J. Syph.*, **XX.**, 124.

ROBERT, A. E., and SAUTON, B., 1916, Action du bismuth sur la spirillose des poules. *Ann. Inst. Past.*, **XXX.**, 261.

SAUNDERS, G. M., 1937, The control of yaws by an intensive treatment method. *Amer. J. trop. Med.*, **XVII.**, 335.

SAZERAC, R., and LEVADITI, C., 1921, Action de certains derivés du bismuth sur la syphilis. *C. R. Acad. Sci.*, **CLXXIII.**, 1201.

DRUG RESISTANT SYPHILIS

Drug resistance on the part of syphilitics is by no means rare; such resistance may be shown only against arsenical preparations, more rarely against arsenic, bismuth and mercury compounds. The question whether resistance is due to a change in the character of the spirochætes, analogous to the drug resistance of trypanosomes or to an idiosyncrasy on the part of the host, has received considerable attention. Some evidence in favour of an acquired resistance to arsphenamine on the part of the spirochæte has been brought forward by Beerman (1936), who found that a strain of spirochæte obtained from a patient with treatment-resistant syphilis was, when inoculated into rabbits, more resistant to arsphenamine than the well-known Nichol's strain of *Spirochæta pallida*. MacCormac (1935) has recorded cases where a patient resistant to arsenic and bismuth has transmitted these characteristics together with the spirochætal infection. On the other hand attempts to produce resistance to neoarsphenamine by repeated passage of spirochætes in rabbits treated with subcurative doses of neoarsphenamine before transfer entirely failed in the hands of Greenbaum and Rule (1936). Occasionally also in cases of conjugal syphilis one partner may respond normally while the other is resistant to arsenical treatment. It thus seems probable that drug resistant syphilis may be due either to idiosyncrasies on the part of the individual or to changes in the strain of spirochæte.

References

BEERMAN, H., 1936, The problem of treatment resistant syphilis with special reference to arsphenamine resistant syphilis : review of the literature and experimental study. *Amer. J. Syph.*, XX., 165.

GREENBAUM, S. S., and RULE, A. M., 1936, Resistant syphilis with especial reference to an attempt to produce experimentally an arsenic-resistant (neoarsphenamine resistant) strain of *Spirochœta pallida*. *Penn. med. J.*, XXXIX., 339.

MACCORMAC, H., 1935, Arsenic resistant syphilis. *Proc. Roy. Soc. Med.*, XXVIII., 1527.

OTHER COMPOUNDS IN THE TREATMENT OF SYPHILIS

In addition to arsenic, bismuth and mercury the following elements have been found to possess spirochæticidal action in rabbits—tellurium, vanadium, platinum, antimony, thorium, strontium, indium, iridium, gallium, gold and rhodium. Rhodium was found by Jahnel (1937 [1]) to produce rapid healing of lesions in rabbit syphilis, but to be irritating when injected intramuscularly and very toxic when given intravenously. Osmium, though therapeutically active in relapsing fever, was without effect in rabbit syphilis (Jahnel, 1937 [2]). Jahnel (1937 [3]) has also investigated the action of ruthenium in experimental syphilis.

Browning and Gulbransen (1936), using as a test-object *Spirillum minus*, by means of which the antispirochætal properties of arsphenamine were originally demonstrated, found that certain anil and styryl quinolines were potentially spirochæticidal, notably 2(*p*-dimethylaminoanil)6-acetylaminoquinolinemethochloride and 2(*p*-methylaminostyryl)acetylaminobenzthiazolemethosulphate.

It seems probable that when the next great advance is made in the chemotherapy of spirochætal infections it will involve the use of compounds free from either metals or metalloids.

References

Browning, C. H., and Gulbransen, R., 1936, Chemotherapeutic action on *Sp. minus* in mice by certain anil and styryl quinoline compounds free from metals or metalloids. *J. Pharmacol.*, LVII., 56.

Jahnel, F., 1937[1], Über die Heilwirkung von Rhodiumverbindungen bei der experimentellen Syphilis und Framboesie. *Klin. Wschr.*, XVI., 657.

Jahnel, F., 1937[2], Über die chemotherapeutische Prüfung einiger Osmiumverbindungen : ihre Unwirksamkeit bei Syphilis, ihre Wirksamkeit bei Recurrens. *Z. Hgy. InfektKr.*, CXIX., 613.

Jahnel, F., 1937[3], Prüfung der chemotherapeutischen Wirkung des Rutheniums bei der experimentellen Syphilis. *Z. ImmunForsch.*, XCI., 312.

CHEMOTHERAPEUTIC PROPHYLAXIS OF SYPHILIS

The prophylaxis of syphilis may be divided into mechanical, chemical and chemotherapeutic (*cf.* Moore, 1937). Experimentally it has been shown by Mahony (1936) that *Spirochæta pallida* does not invade the mucous membrane of the penis for from two to three hours after it has been placed on the surface. During this interval it can be removed quite easily by soap and water or destroyed by a number of chemicals in solution, more especially when the *p*H of these solutions is on the acid side of neutrality.

True chemotherapeutic prophylaxis was first attempted by Sazerac and Levaditi (1922), who found that injections of bismuth prevent syphilitic infection in rabbits. Levaditi and Vaisman (1937) believe that injections of bismuth subnitrate given at intervals of fifteen or sixteen days protect rabbits for as long as 154 days after injection. Tellurium (Levaditi *et al.*, 1928), gold (Levaditi *et al.*, 1934) and quinquevalent arsenic in the form of acetarsol (Levaditi *et al.*, 1922) also exercise a certain degree of prophylactic action in rabbits, but are less active than bismuth.

Bismuth has been used in man for the prevention of syphilis. Sonnenberg (1935) injected 2·5 c.c. of a 10 per cent. suspension of bismuth subnitrate in oil either once or more frequently twice a week into 327 prostitutes. Only 27 of these acquired syphilis, in every instance before treatment had gone on for as long as fourteen weeks. Ramijean and Tran-Tan-Phat (1937) also used bismuth salts in prophylaxis. Fifty-seven prostitutes were given daily intramuscular injections and at the end of eleven months none had contracted syphilis, while of 28 controls 15 had acquired syphilis in the same period.

These remarkable results have not, unfortunately, been universally obtained, and in practice the treatment is not easily applied (*cf.* Rabut, 1936, and Gaté and Cuilleret, 1936).

Treatment of the mother does not necessarily protect the fœtus, since Vámos and Böhm (1937) have found that in rabbits arsphenamine is deposited in the intervillous spaces and the vessels of the decidua but not in the chorion nor in any of the fœtal tissues.

References

GATÉ, J., and CUILLERET, P., 1936, À propos de la métallo-prévention par le bismuth. *Bull. Soc. franç. Derm. Syph.*, **XLIII.**, 1191.

LEVADITI, C., NAVARRO-MARTIN, L., FOURNIER, L., GUÉNOT, L., and SCHWARTZ, A., 1922, Recherches sur l'action curative et préventive de l'acide acétyloxyaminophénylarsinique (190 ou stovarsol) administré par voie digestive dans la syphilis. *Ann. Inst. Past.*, **XXXVI.**, 729.

LEVADITI, C., SANCHIS-BAYARRI, V., SCHOEN, R., and MANIN, Y., 1928, Métallo-prévention de la syphilis. *Ibid.*, **XLII.**, 105.

LEVADITI, C., and VAISMAN, A., 1937, Métallo-prévention bismuthique de la syphilis. *Bull. Soc. Path. exot.*, **XXX.**, 849.

LEVADITI, C., VAISMAN, A., KRASSNOFF, D., and SCHOEN, R., 1934, La métallo-prévention de la syphilis au moyen des dérivés de l'or hydrosolubles et liposolubles. *Bull. Acad. Méd. Paris*, **CXI.**, 215.

MAHONY, J. F., 1936, An experimental resurvey of the basic factors concerned in prophylaxis in syphilis. *Milit. Surg.*, **LXXVIII.**, 351.

MOORE, J. E., 1937, Prophylaxis in the control of syphilis. *Sth. med. J.*, **XXX.**, 149.

RABUT, R., 1936, À propos du traitement préventif de la syphilis. *Bull. Soc. franç. Derm. Syph.*, **XLIII.**, 1186.

RAMIJEAN, R., and TRAN-TAN-PHAT, 1937, La métallo-prévention dans la syphilis : résultats obtenus au dispensaire antivénérien régional de Saigon-Cholon. *Bull. Soc. méd.-chir. Indochine*, **XV.**, 362.

SAZERAC, R., and LEVADITI, C., 1922, Emploi du bismuth dans la prophylaxie de la syphilis. *C. R. Acad. Sci.*, **CLXXIV.**, 128.

SONNENBERG, E., 1935, Neuf ans de traitement préventif bismuthique de la syphilis. *Bull. Acad. Méd. Paris*, **CXIV.**, 374.

VÁMOS, J., and BÖHM, A., 1937, Die Wirkung der Arsenobenzolpräparate auf den Fetus (Tierversuche). *Arch. Dermat. u. Syph.*, **CLXXVI.**, 245.

CHAPTER VII

THE CHEMOTHERAPY OF TUBERCULOSIS

In the treatment of leprosy the action of chaulmoogra, though unexplained, is probably to a certain degree specific : no chemotherapeutic agent, however, has yet been found with anything approaching a specific action in tuberculosis. One of the main difficulties in discovering such a specific agent for tuberculosis lies in the fact that all drugs must be tested on man, for the experimental tuberculosis of small rodents is so acute a disease that it differs radically from the more chronic reaction in man. Nevertheless, two main lines of chemotherapeutic treatment have been attempted by (i) calcium salts, (ii) salts of gold and other heavy metals.

CALCIUM SALTS

Since calcification appears to be associated with the healing of tuberculous foci, it is perhaps natural that attempts should have been made to increase the rapidity of the healing process by the administration of calcium salts with or without the addition of vitamin D or parathyroid extract. Unfortunately, there is little evidence to show that prolonged administration of calcium increases the blood calcium. Teplitz (1925), for instance, in cases of phthisis with normal blood calcium figures, found no definite increase after three months' treatment with calcium lactate and, as Maver and Wells (1923) point out, in tuberculosis " there is no evidence that calcification follows, rather than precedes, the destruction of the tubercle bacilli."

Steiner, Greene and Kramer (1937) did not find that a diet lacking vitamin D had any effect on experimental tuberculosis of the rabbit, while Steenken and Baldwin (1937) could not determine any beneficial effects from a diet containing irradiated milk or oils containing vitamin D.

Cod liver oil has long been believed to possess a beneficial effect in tuberculosis, but proof that any such effect is due to its vitamin A or D content is lacking. Getz (1938) in fact concludes that the healing factor is not one of these vitamins. Since, however, chaulmoogric and hydnocarpic acids have an inhibitory action on leprosy bacilli, sodium morrhuate has also been tested for an inhibitory action on tubercle bacilli. Rogers (1933) obtained improvement in cases of lupus by intradermal inoculation of the ethyl esters of hydnocarpus oil with the addition of 4 per cent. creosote. The injections, however, caused considerable pain and local reaction. In order to overcome this irritation Burgess (1935) employed intradermal injections into the lesions of phenyl ethyl hydnocarpate, with the result that localized patches were healed in seven out of eleven patients.

Further observations are required before the full value of this treatment can be determined.

SALTS OF GOLD AND OTHER HEAVY METALS

During the nineteenth century it was discovered that certain simple gold salts, such as gold and potassium cyanide and gold chloride, inhibited the growth of tubercle bacilli *in vitro*. Gold chloride, however, had no action in controlling experimental tuberculosis in animals. Later complex organic gold compounds, such as the sodium salt of *p*-amino-*o*-auromercáptobenzenecarboxylic acid, were introduced and, though the results obtained in animals were disappointing, improvement was claimed in human cased (Feldt, 1924).

In 1924 Møllgaard introduced under the name of " sanocrysin " the double thiosulphate of gold and sodium,

[illegible]

(also known as " crysalbine "), a compound originally prepared in 1845 by Fordos and Gélis. A number of other gold preparations have since been used in the therapy of human tuberculosis and also in the treatment of rheumatoid arthritis. The most important of these compounds are :—

Sodium aurothiomalate	"myocrysin."
Sodium aurothiopropanol sulphonate and Sodium thiopropanolsulphonate	"allochrysine."
Sodium aurothioglucose	"solganal B"
Disodium *p*-sulphomethylamino-*o*-aurothio benzenesulphonic acid	"solganal."
Sodium aurothiosinamine benzoic acid	"lopion."
Sodium 2-aurothiobenziminazole-4-carboxylate	"triphal."
Sodium-*p*-*N*-dimethylamino-*o*-tolylphosphinate sodium aurothiosulphate	"aurophos."

"Auro-detoxin" is said to be a colloidal compound of gold with derivatives of keratin analogous to polypeptides, containing 12 per cent. of metallic gold. Collier and Verhoog (1937) claim to have obtained good results in experimental tuberculosis. Balzer and Dosquet (1937) have treated 55 human cases.

An exact comparison of the therapeutic effects of these various gold preparations has not been made, although "solganal" is said to be less irritating to the kidneys than "sanocrysin." As the water soluble salts of gold are tissue irritants it is necessary to inject them intravenously : gold salts insoluble in water may, however, be given intramuscularly if suspended in oil. In these circumstances, however, as much as 50 per cent. of the compound may, according to Leulier and Payre-Ficot (1934), remain at the site of inoculation. Certain observers, such as Schröder (1933), believe that "sanocrysin" and "solganal B" in oil are less toxic and more efficient than aqueous preparations. After injection gold salts are rapidly taken up by the reticulo-endothelial cells, particularly those of the liver and spleen. Traces of gold are also found in the pancreas, heart, lungs and skin, as well as in tuberculous foci. Although the concentration of gold present in tuberculous tissue is often greater than that necessary to inhibit the growth of tubercle bacilli *in vitro*, the distribution of gold in normal and diseased tissues is approximately equal. Lebeuf, Mollard and Pauget (1931) found gold in the cerebro-spinal fluid, while Steiner and Fischl (1929) found small quantities in the brains of dogs and large amounts in the grey matter of a man with disseminated sclerosis.

In therapeutic doses gold salts tend to cause a leucocytosis with increase in the number of lymphocytes.

The somewhat over-optimistic hopes which were at first entertained of the chemotherapeutic action of " sanocrysin " in human tuberculosis have unfortunately not been fulfilled, but on the other hand it is now possible to avoid the severe reactions which at first caused a number of deaths owing to the acute poisoning produced by the large doses that were given at intervals of three or four days.

It is now realized that the margin between therapeutic effect and toxic action is small and that certain persons are curiously sensitive to the injection of gold compounds, though Copeman and Tegner (1937) have found that as a rule patients with tuberculosis are less susceptible to gold than those with arthritic conditions. It is therefore essential that only very carefully selected cases should be subjected to gold therapy, and that the effects of the injections should be carefully watched by constant examinations of the urine for traces of albumen, and of the red cell sedimentation rate, which with improvement should show a reduction (*cf.* Patterson, 1936). Throne *et al.* (1932) state that a reduction of blood chlorides and an increase of blood sugar frequently accompany the onset of toxic symptoms. An increase of polymorphonuclear leucocytes or eosinophils and a diminution of blood platelets all contra-indicate the continuance of gold therapy.

In most instances toxic reactions occur after a small number of gold injections, the limit of physiological tolerance being rapidly reached; occasionally, however, the onset of symptoms may be much delayed. In rabbits Vallery-Radot, Gilbrin and Gauthier-Villars (1935) found that toxic doses of " allochrysine " and " sanocrysin " may either cause death or induce tolerance. The immediate result of injection of gold salts into rabbits was to cause the appearance of albumen and casts in the urine associated with increased blood urea. Histologically metallic granules first appeared in rows along the basement cells of the convoluted tubules, while the cells of the convoluted tubules were normal. Later the granules entered the cells and were excreted into the lumen, and at this stage the tubular cells had become swollen and necrotic. The glomeruli were unaffected and very few granules were present in the liver. Kálló (1934) obtained slight parenchymatous changes in the kidneys of rabbits, rats and guinea-pigs even with therapeutic doses. In human beings toxic reactions caused by the injection of gold salts are by no means infrequent. An analysis of 400 cases by Hartfall and Garland (1936) revealed toxic reactions in 37 per cent. The reactions may be classified as follows :—

(1) Febrile : occasionally associated with anaphylactoid symptoms.

(2) Gastro-intestinal : loss of appetite, nausea, vomiting, diarrhœa, stomatitis, with ulcers on the lips and mouth.

(3) Circulatory : fall of blood pressure and collapse.

(4) Hæmopoietic : polymorphonuclear leucocytosis with eosinophilia ; agranulocytosis ; thrombopenia with purpura hæmorrhagica (*cf.* Secher, 1934). Witts (1936) and Heinild (1937) regard the association of thrombopenia with "sanocrysin" as most probably allergic.

(5) Hepatic : toxic jaundice.

(6) Renal : albuminuria, hæmorrhagic nephritis, anuria.

(7) Arthritic : acute arthritis.

(8) Cutaneous : rashes, morbilliform, erythematous and purpuric ; exfoliative dermatitis—Hudson (1935) collected accounts of 22 cases. In certain instances pigmentation of the skin may develop, the symptoms being not unlike those of argyria ; this aurodermatitis or chrysoderma is often very persistent ; blondes suffer more than brunettes, the pigmentation being specially marked in parts exposed to light. The pigmentation is intensified by exposure to ultra-violet light and is therefore most marked in summer.

(9) Nervous. Although among 148 cases of reactions to gold salts Hartfall and Garland (1936) did not record any with involvement of the central nervous system, nevertheless a not inconsiderable number with such involvement have been reported,

more especially in France. Not infrequently the nervous symptoms may be accompanied by involvement of the skin. Lescher (1936) groups the nervous phenomena as follows :—

(*a*) Generalized, with mental symptoms predominant. The symptoms may consist of generalized stabbing pains with cutaneous hyperæsthesia, fever (grippe aurique) with a minimum of objective signs; mental agitation associated with extreme restlessness at night and in the early morning, depression and morbid fears; insomnia and great fatigue; excessive sweating and loss of flesh; irregular fibrillary twitchings of the muscles of the trunk and limbs (Hinault and Mollard, 1934).

(*b*) Peripheral nerves (*cf.* Roger, Alliez and Jouve, 1935): weakness and pain on pressure over the calves; severe sciatica, peripheral neuritis and paresis of limbs. The cerebro-spinal fluid may show an increase of lymphocytes and of protein.

(*c*) Paralysis of Landry's type. Bourgeois, Thiel and Broutman (1935) record a case of flaccid paraplegia followed by paralysis of the right upper limb, bulbar paralysis and death.

(*d*) Epileptiform convulsions.

(*e*) Meningitis, as a result of activation of a tuberculous focus with extension to meninges (Berthier, Rouit and Offant, 1932).

Fortunately the prognosis in nervous complications is usually good, except in patients with mental disturbance and in those with tuberculous meningitis.

In view of what has been said in regard to reactions it is obvious that gold salts should not be administered to persons with gastro-intestinal or renal lesions or with tuberculous foci which might conceivably spread to the meninges. They are also best avoided in patients with recurrent attacks of hæmoptysis, great emaciation or toxic symptoms with high fever.

The cases which alone are suitable for gold therapy are, according to Banerjea (1937), those with recent exudative lesions. With discretion chrysotherapy may be applied to (1) cases where it is desirable to collapse one lung by artificial pneumothorax or phrenic evulsion and to check the progress of recent lesions in the other lung; (2) cases of a chronic fibrotic type with acute exacerbations. In place of the comparatively large doses formerly given, it is now usual to begin with small doses of gold salts. Thus Banerjea (1937) in febrile cases begins with intramuscular injections of 0·002 gm. of "solganal B," "oleosum" or "sanocrysin" in oil and increases by 0·002 gm., the injections being given every four to six days. In febrile cases an initial dose of 0·01 gm. may be given and the amount doubled till 0·4 gm. is reached: a total dosage of 5 to 6 gm. should be administered. Other observers give weekly injections of from 0·05 to 0·1 gm., the total dosage not exceeding 1 gm. When this has been attained an interval of at least twelve weeks should elapse.

Opinions are still divided as to the value of gold therapy in tuberculosis. The fact that the kidneys are almost invariably damaged by gold, even though no evidence of the damage may appear in the urine, has caused American workers, such as Amberson, McMahon and Pinner (1931), to be sceptical of its value. In England, Peters and Short (1935), as a result of a controlled statistical study over a period of five years, concluded that in the age group sixteen to twenty-six years chrysotherapy was of no appreciable value. On the other hand, Bernard (1932) claimed improvement in approximately 50 per cent. of cases, while Mayer (1934) obtained good results in 66 per cent. of 404 cases. Terrill (1936) found that of 41 patients treated for a year and a half 28 were improved, 10 of them markedly; 10 patients failed to improve and 3 became worse. In 10 the sputum remained negative. Banerjea (1937) noted improvement in just over half of 60 selected cases: the disappearance of tubercle bacilli from the sputum was, as a rule, only temporary. Frimodt-Möller and Verghese (1937) reported that of 139 patients with pneumothorax who were given "sanocrysin" 89, or 64 per cent., improved, while of 168 patients with pneumothorax but without "sanocrysin" only 61, or 36 per cent., improved.

While gold is far from having a specific action on tubercle bacilli, the fact that it is still being used in many countries after a trial of several years is sufficient reason to believe that it is possessed of some efficacy.

In primary acute cases gold salts usually fail to produce any effect whatever, and in chronic pulmonary tuberculosis they fail to prevent recurrent relapses. They may, however, check exacerbation of the disease and bring about a period of arrest. Since observations have shown that the growth of tubercle bacilli *in vitro* is not inhibited by quite large concentrations of " sanocrysin " (Fry, 1926) the action of gold salts has been attributed to a stimulation of the defence mechanism of the body. Russu and Sichet (1937), for instance, have shown that under the influence of " sanocrysin " the phagocytic activity of the reticulo-endothelial cells is greatly increased.

Recently Japanese workers have suggested that the value of the various gold preparations employed in the treatment of human tuberculosis is due not so much to the gold they contain as to the presence of the sulphydryl group in the form of the aurothio (AuS), thiosulphate or sulphite group.

Sodium thiosulphate was originally used experimentally and clinically by Xalabarder and Alegret (1933), whose results have been confirmed by Yanagisawa (1936). More recently Yanagisawa and Kawai (1937) have shown that while sodium aurothiophenol-*m*-carboxyl and sodium thiophenol-*m*-carboxyl inhibit to about the same extent the development of experimental tuberculosis in the guinea-pig, sodium dithiophenol-*m*-carbonate has no such retarding influence. Further observations are obviously required on the effect of the sulphydryl group on the growth of tubercle bacilli.

In view of the apparently beneficial action of gold in certain cases of tuberculosis the effect of a number of other heavy metals has been tested. Walbum (1926), for instance, found that in experimental tuberculosis of rodents an inhibitory action was exerted by aluminium, barium, cadmium, cerium, lanthanum, molybdenum, ruthenium and selenium : the inhibitory action of platinum and iridium was less definite. Menkin (1937) suggested that repeated intravenous or intramuscular injections of ferric chloride produced beneficial results, while Mercader (1934) and Stephani (1934) have used colloidal copper morrhuate and organic copper derivatives such as copper benzoate.

Cadmium, first employed by Lund (1927), has more recently been tested by Maigre and Reynier (1932) in the form of an iodine-gold-cadmium sulphide or glycine. It is non-toxic and painless and in animals appears to stimulate the growth of fibrous tissue. Heaf (1937) found that of twenty patients who received intramuscular injections of 3 c.c. of a 1 per cent. colloidal cadmium suspension four became clinically quiescent, seventeen improved, two remained stationary and only two became worse. The results were thus equal to those obtained with gold : the absence of toxic reaction and the lowered cost were in favour of cadmium.

Rich and Follis (1938) observed marked retardation in the progress of experimental tuberculosis in guinea-pigs treated with large doses of sulphanilamide, but these results lack complete confirmation (Smithburn, 1938, and Buttle and Parish, 1938).

References

AMBERSON, J. B., McMAHON, B. T., and PINNER, T., 1931, A clinical trial of sanocrysin in pulmonary tuberculosis. *Amer. Rev. Tuberc.*, **XXIV., 401.**

BALZER, H., and DOSQUET, H., 1937, Zur Behandlung der Lungentuberkulose. *Deut. Tub. Blatt.*, XI., 60.

BANERJEA, J. C., 1937, Some observations on chrysotherapy in pulmonary cases. *Indian med. Gaz.*, LXXII., 205.

BERNARD, L., 1932, Chrysothérapie et pneumothorax artificiel. *Pr. méd.*, XL., 1901.

BERTHIER, J., ROUIT and OFFANT, 1932, Chrysothérapie, nettoyage radiologique de lésions tuberculeuses chez une femme enceinte, apparition ultérieure d'une méningite tuberculeuse. *Bull. Soc. Méd. Marseille* (June 27th).

BOURGEOIS, P., THIEL, H., and BROUTMAN, J., 1935, Accident mortel de la chrysothérapie : syndrome de Landry associé à une erythrodermie aurique. *Bull. Mém. Soc. méd. Hôp. Paris*, LI., 90.

BURGESS, N., 1935, Lupus vulgaris. A note on a new method of treatment by intradermal injection of phenyl ethyl hydnocarpate. *Brit. Med. Journ.*, *ii.*, 835.

BUTTLE, G. A. H., 1938, Treatment of tuberculosis in guinea-pigs with sulphanilamide. *Brit. med. J.*, *ii.*, 776.

COLLIER, W. A., and VERHOOG, M. J., 1937, Die Wirkung von Auro-Detoxin und Auro-Detoxin Type 70 auf verschiedene Infektionen. *Z. Immun-Forsch.*, XC., 174.

COPEMAN, W. S. C., and TEGNER, W., 1937, A review of gold therapy. *Lancet*, *i.*, 554.

FELDT, A., 1924, "Die Goldbehandlung der Tuberkulose und der Lepra," Halle.

FORDOS, M. J., and GÉLIS, A., 1845. Action du perchlorure d'or sur l'hyposulfite de soude. *Ann. Chim.* (*Phys.*), XIII., 394.

FRIMODT-MÖLLER, C., and VERGHESE, M. C., 1937, An analysis of artificial pneumothorax treatment in 1,039 patients. *Indian med. Gaz.*, LXXII., 208.

FRY, R. M., 1926, The effect of sanocrysin on *B. tuberculosis*. *Brit. J. exp. Path.*, VII., 174.

GETZ, H. R., 1938, Cod liver oil therapy in experimental tuberculosis. *Proc. Soc. exp. Biol. N.Y.*, XXXVIII., 543.

HARTFALL, S. J., and GARLAND, H. G., 1936, Further observations on the gold treatment of rheumatoid arthritis. *Lancet, i.*, 1459.

HEAF, F., 1937, The treatment of tuberculosis by heavy metals, excluding gold, but with particular reference to the use of cadmium. *Brit. J. Tub.*, XXXI., 66.

HEINILD, S., 1937, Problems concerning the behaviour of thrombocytes during sanocrysin therapy. *Acta med. Scand.*, XCII., 308.

HINAULT, V., and MOLLARD, H., 1934, " La traitement aurique de la tuberculose." Paris : Vigot Frères.

HUDSON, E. H., 1935, Purpura hæmorrhagica caused by gold and arsenical compounds. *Lancet, ii.*, 74.

KÁLLÓ, A., 1934, Lésions rénales causées par les sels d'or. *Ann. Anat. path.*, XI., 21.

LEBEUF, F., MOLLARD, H., and PAUGET, P., 1931, Présence de l'or dans le liquide céphalo-rachidien des sujets traités par les injections intraveineuses de crisalbine. *Bull. Soc. franç. Dermat. et Syph.*, XXXVIII., 513.

LESCHER, F. G., 1936, Nervous complications following treatment by gold salts. *Brit. Med. Journ., ii.*, 1303.

LEULIER, A., and PAYRE-FICOT, L., 1934, Sur le sort de quelques composés de l'or dans l'organisme animal. *C. R. Soc. Biol. Paris*, CXVI., 530.

LUND, N., 1927, Behandlung von Lungentuberkulose mit Metallsalzen nach der vom Dr. Walbum angegebenen Methode. *Z. f. Tub.*, XLVIII., 286.

MAIGRE, E., and REYNIER, P., 1932, Sur le traitement de la tuberculose par un complexe organo-métallique à base d'iode, de cadmium et d'or. *Rev. Méd.*, XLIX., 174.

MAVER, M. E., and WELLS, H. G., 1923, The absorption of calcium and its deposition in the tissues of experimental tuberculosis. *Amer. Rev. Tuberc.*, VII., 1.

MAYER, C., 1934, Gold treatment of pulmonary tuberculosis. *Brit. J. Tuberc.*, XXVIII., 131.

MENKIN, V., 1937, Tolerance tests with ferric chloride on advanced tuberculosis patients. *Amer. Rev. Tuberc.*, XXXV., 134.

MERCADER, N., 1934, El gadusán en la tuberculosis. *Sem. méd.*, I., 1749.

MØLLGAARD, H., 1924, " Chemotherapy of tuberculosis," Copenhagen.

MØLLGAARD, H., 1925, The theoretical basis of the sanocrysin treatment of tuberculosis. *Brit. Med. Journ., i.*, 643.

PATTERSON, H. A., 1936, Observations on the red-cell sedimentation test in pulmonary tuberculosis. *Amer. Rev. Tuberc.*, XXXIV., 164.

PETERS, B. A., and SHORT, C., 1935, Gold treatment of tuberculosis : a statistical study. *Lancet, ii.*, 11.

RICH, A. R., and FOLLIS, R. H., 1938, The inhibitory effect of sulfanilamide in the development of experimental tuberculosis in the guinea-pig. *Bull. Johns Hopk. Hosp.*, LXII., 77.

ROGER, H., ALLIEZ, J., and JOUVE, A., 1935, Etude clinique et physiopatholoqique des accidents nerveux de l'aurothérapie. *Arch. Méd. gén. colon.* IV., 145.

ROGERS, L., 1933, Observations on the use of hydnocarpates and morrhuates intradermally in lupus vulgaris. *Brit. Med. Journ., i.*, 47.

RUSSU, V., and SICHET, P., 1937, Der Einfluss des Sanocrysins auf das retikulo-endotheliale System im gesunden und tuberkulösen Organismus. *Münch. med. Wschr.*, LXXXIV., 186.

SCHRÖDER, G., 1933, Zur Goldtherapie der Tuberkulose. *Tubercle*, XIV., 193.

SECHER, K., 1934, Sanocrysin problems : thrombopenia during sanocrysin treatment. *Acta tub. Scand.*, VIII., 120.

SMITHBURN, K. C., 1938, Inefficacy of prontylin in experimental tuberculosis. *Proc. Soc. exp. Biol. N.Y.*, XXXVIII., 574.

Steenken, Jr., W., and Baldwin, E. R., 1937, The effect of irradiated milk compared with vitamin D oils on inhalation tuberculosis of guinea-pigs. *Amer. Rev. Tuberc.*, XXXV., 656.

Steiner, G., and Fischl, V., 1929, Experimentelle Untersuchungen zur Pathologie und Therapie der Spirochätenkrankheiten. Uber die Wirkung von Goldpräparaten bei experimenteller Recurrens. *Klin. Wschr. Berlin*, VIII., 582.

Steiner, M., Greene, M. R., and Kramer, B., 1937, The effect of vitamin D deficiency on experimental tuberculosis in the rabbit. *Amer. Rev. Tuberc.*, XXXV., 640.

Stephani, T., 1934, Traitement de la tuberculose par les dérivés benzéniques iodés du cuivre et du didyme. *Rev. Tuberc.*, II., 253.

Teplitz, M. M., 1925, The effect of the oral administration of calcium lactate and thyroid extract on the calcium content of the blood serum in pulmonary tuberculosis. *Amer. Rev. Tuberc.*, XII., 222.

Terrill, F. I., 1936, Results of sanocrysin treatment of tuberculosis. *Amer. Rev. Tuberc.*, XXXIV., 156.

Throne, B., Kingsbury, J., and Myers, C. N., 1932, Unusual clinical manifestations following intravenous administration of gold compounds. *Arch. Dermat. and Syph.*, XXV., 494.

Vallery-Radot, P., Gilbrin, E., and Gauthier-Villars, P., 1935, Néphrites expérimentales par les sels d'or. Parallélisme entre l'élimination du metal et les lésions histologiques. *Ann. Méd.*, XXXVII., 145.

Walbum, L. E., 1926, Metallsalztherapie. *Z. ImmunForsch.*, XLVII., 213.

Witts, L. J., 1936, Effect of toxic substances on the blood-forming organs with special reference to therapeutic drugs. *Brit. Med. Journ.*, *ii.*, 211.

Xalabarder, C., and Alegret, E., 1933, Investigación sobre la acción del tiosulfato sódico en la tuberculosis ; datos experimentales. *Rev. Hig. y. Tub.*, XXVI., 319.

Yanagisawa, K., 1936, On the influence of inorganic reducing substances on experimental tuberculosis. *Japan J. exp. Med.*, XIV., 395.

Yanagisawa, K., and Kawai, S., 1937, The significance of the gold molecule of the gold compounds in the chemotherapy of tuberculosis. *Jap. J. exp. Med.*, XV., 29.

CHAPTER VIII

THE CHEMOTHERAPY OF LEPROSY

THE modern treatment of leprosy dates from 1897, when, as a result of the First International Scientific Congress on Leprosy held in Berlin, the theory of hereditary transmission was finally rejected and the old mediæval belief that leprosy was contagious was re-affirmed. Any estimation of the value of treatment in leprosy is fraught with considerable difficulties, owing to the seemingly spontaneous periods of quiescence and activity apart from treatment, the fact that most patients first report sick during an exacerbation which subsides spontaneously, the influence on the course of the illness of season, climate, diet and intercurrent disease, and the inability to find a lower animal in which *Mycobacterium lepræ* will produce a condition resembling that seen in man. The recent demonstration by Adler (1937) that the Syrian hamster (*Cricetus auratus*) is susceptible to human leprosy may provide such an experimental animal.

It is, therefore, very difficult to say that any one drug or method of treatment has succeeded in curing a case of leprosy, for even though no signs of active disease are present and the patient may apparently be free from active lesions for several years, recurrence is liable to take place as a result of any lowering of the general resistance. It is thus of the greatest importance, as Muir (1931) has repeatedly emphasized, that the general resistance should be maintained at as high a level as possible and that, apart from specific treatment, the patient should enjoy ample diet and freedom from worry, while intercurrent diseases such as malaria, syphilis and ankylostomiasis should also receive attention.

The number of drugs that have been used in time past for the treatment of leprosy is very great. At present, however, the chemotherapeutic treatment of leprosy is dependent on :—

(i.) Certain fatty acids and their derivatives.
(ii.) Metallic preparations.
(iii.) Aniline dyes.

CHAULMOOGRA AND HYDNOCARPUS OILS

Chaulmoogra oil has been used in the treatment of leprosy for many centuries in India, but it was first introduced into western medicine by Mouat (1854), who reported improvement in a case of leprosy following the oral administration and local application of chaulmoogra.

Up till 1900 it was believed that chaulmoogra oil was derived from the seeds of *Gynocardia odorata,* R. Brown. Prain (1901), however, showed that true chaulmoogra oil was obtained from the seeds of *Taraktogenos kurzii,* King, a tree grown in Burma and Assam. The seeds of *Taraktogenos kurzii,* though very similar to those of *Gynocardia odorata,* may be distinguished by the fact that the radicle of the seed is terminal, while in *Gynocardia* it is lateral.

In addition to *Taraktogenos kurzii* a number of other trees belonging to the Flacourtiaceæ yield seed oils with a composition akin to that of true chaulmoogra oil. The Table on p. 300 records the chief constants of some of these : the most important are :—

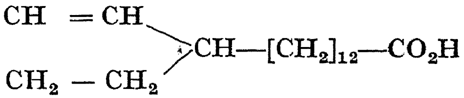
	Habitat
Hydnocarpus Wightiana Blume.	Malabar coast of India.
H. anthelmintica Pierre.	Siam, French Indo-China.
Carpotroche brasiliensis Endl.	Brazil.

Although chaulmoogra oil is the traditional material for the treatment of leprosy, it has now been largely replaced by *Hydnocarpus Wightiana* oil which is at least as strongly active optically, contains the same constituents, has a lower acid number and is more readily obtainable as the trees occur in more accessible areas. The successful cultivation of *H. Wightiana* and *H. anthelmintica* trees from seeds distributed by the British Empire Leprosy Relief Association has now been reported from tropical Africa, British Guiana, the West Indies and Fiji.

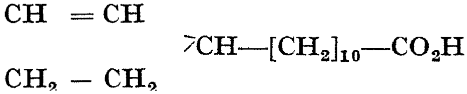
CHARACTERISTICS OF THE CHAULMOOGRA AND ALLIED OILS.

Oil.	Specific Gravity.	Refractive Index $n \frac{30^\circ}{D}$.	Freezing-Point °C.	Rotation 100 mm. 30°/D.	Iodine Number Hanus.	Saponification Number.	Acidity as per cent. Oleic.	Fatty Acids Freezing-Point °C.	Fatty Acid Specific Rotatory Power $[\alpha] \frac{30^\circ}{D}$.
Gynocardia odorata	0·929 (30°)	1·4743	4	0	160·0	198	2·7	20	0
Hydnocarpus alcalæ	0·948 (30°)	1.4763	24	48·3	94·0	202	6·7	55	40
H. anthelmintica	0·952 (30°)	1.4630	16	44·2	84·5	201	3·6	36	50
H. Hutchinsonii	0·943 (30°)	1.4743	23	44·0	83·5	199	5·3	43	50
H. subfalcata	0·951 (30°)	1·4761	21	49·1	89·0	206	6·6	41	36
H. venenata	0·947 (30°)	1·4769	20	46·4	90·7	191	1·2	47	49
H. Wightiana	0·947 (30°)	1·4763	11	51·2	97·0	207	6·7	40	54
H. Woodii	—	1·4730	18	45·9	68·5	192	5·9	43	53
Taraktogenos Kurzii	0·951 (30°)	1·4771	9	43·5	104·0	215	3·4	32	43
Asteriastigma macrocarpa	0·955 (30°)	—	—	48·1	95·2	198	—	—	—
Carpotroche brasiliensis Paget (1937)	0·9563 (25°)	—	—	54·0	101·3	199·7	21·7	—	—
Gonsalves (1934)	0·9584 (20°)	1·478 (27°)	12	51·2	111·7	198·8	6·53	—	—
	0·9577 (20°)	1·478 (27°)	9	51·5	108·1	200·7	15·86	—	—

In order to be of satisfactory quality *Hydnocarpus Wightiana* oil should conform to the following standards laid down by the British Pharmacopœia, 1932. The oil should be partially insoluble in cold alcohol (90 per cent.) and almost wholly soluble in hot alcohol (90 per cent.). Sp. G. (25°/25°) 0·950 to 0·960: melting-point 20° to 25° C. Specific rotation, in a solution made by dissolving 10 gm. of oil in chloroform and diluting to 100 c.c. with the same solvent, should be not less than + 53°; refractive index at 40°, 1·472 to 1·476; acid value not more than 25; saponification value 198 to 304; iodine value 97 to 103. The first oils of this type to be fully examined were chaulmoogra, *H. Wightiana* and *H. anthelmintica* oils (Power and Gornall, 1904, and Power and Barrowcliff, 1905). These oils contain principally glycerides of the characteristic homologous acids, chaulmoogric, $C_{18}H_{32}O_2$, and hydnocarpic, $C_{16}H_{28}O_2$, which were shown by these investigators to have the following constitution :—

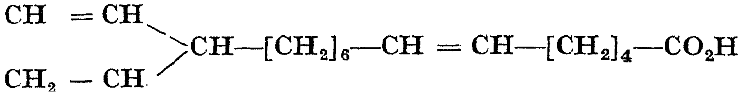


Chaulmoogric acid.



Hydnocarpic acid.

Chaulmoogric acid melts at 68·5°, $[\alpha]^{25°}_{D}$ + 60·3°, iodine absorption value (Hanus) 90·5. Hydnocarpic acid melts at 60·5°, $[\alpha]^{25°}_{D}$ + 69·3°, iodine absorption value 100·7. Small quantities of oleic, palmitic and lauric acids have also been found (Cole, 1929) and evidence obtained of a more highly unsaturated acid (Wrenshall and Dean, 1924) which is probably dehydrochaulmoogric acid (see below). Da Silva (1926) and Rothe and Surerus (1931) identified chaulmoogric and hydnocarpic acids as the chief constituents of sapucainha (*Carpotroche brasiliensis*) oil and more recently Paget (1937) has found in addition to these, ketochaulmoogric and ketohydnocarpic acids and dehydrochaulmoogric acid, $C_{18}H_{30}O_2$, which contains a second ethylenic linkage



Dehydrochaulmoogric acid.

Gorli seed (*Caloncoba echinata*) oil differs from those already mentioned in containing no hydnocarpic acid (Goulding and Akers, 1913, and André and Jouatte, 1928), a fact which may account for the unfavourable clinical results obtained by Philippine workers with gorli oil and confirmed by Advier and Peirier (1930).

References

Adler, S., 1937, Inoculation of human leprosy into Syrian hamster. *Lancet*, *ii.*, 714.

Advier and Peirier, 1930, Mode d'action des huiles de *Caloncoba* et de leurs dérivés sur les bacilles acido-résistants. *Bull. Soc. Path. exot.*, XXIII., 767.

André, E., and Jouatte, D., 1928, Contribution à l'étude des huiles chaulmoogriques. Recherches sur l'huile de Gorli. *Bull. Soc. chim. Fr.*, XLIII., 347.

Cole, H. I., 1929, The constituents of *Hydnocarpus Wightiana* oil. I. *Philipp. J. Sci.*, XL., 499.

Da Silva, R. A. D., 1926, O chaulmoograto de ethylio. *Rev. brasil. Med. Pharm.*, II., 399.

Goulding, E., and Akers, N. C., 1913, Note on the fat of the seeds of *Oncoba echinata:* occurrence of chaulmoogric acid. *Proc. chem. Soc.*, CIII., 197.

Mouat, F. J., 1854, Notes on native remedies. No. 1, The Chaulmoogra. *Indian Ann. med. Sci.*, I., 646.

Muir, E., 1931, The treatment of leprosy. *Trans. R. Soc. trop. Med. Hyg.*, XXV., 87.

Paget, H., 1937, The liquid acids of sapucainha oil. *J. chem. Soc.*, LXIX., 955.

Power, F. B., and Barrowcliff, M., 1905, The constituents of the seeds of *Hydnocarpus Wightiana* and of *Hydnocarpus anthelmintica.* Isolation of a homologue of chaulmoogric acid. *J. chem. Soc.*, LXXXVII., 884.

Power, F. B., and Gornall, F. H., 1904, The constituents of chaulmoogra seeds. *J. chem. Soc.*, LXXXV., 838.

Prain, D., 1901, Chaulmugra seed. *Pharm. J.*, LXVI., 596.

Rothe, O., and Surerus, D., 1931, Reconhecimento do acido chaulmoogrico no oleo de *Carpotroche brasiliensis*. *Rev. Soc. brasil. Chim.*, II., 358.

Wrenshall, R., and Dean, A. L., 1924, Fractionation of chaulmoogra oil. Evidence of the existence of a highly unsaturated optically active acid. *U.S. Publ. Health Serv. Bull.*, CXLI., 12.

THE PHARMACOLOGY AND TOXICITY OF CHAULMOOGRA PREPARATIONS

Although the term " Chaulmoogra oil " is usually applied to oils obtained from *Taraktogenos kurzii* and certain species of *Hydnocarpus*, there seems little reason why the sapucainha oil of Brazil should not also be included under the term " chaulmoogra," for not only is it chemically very similar, but its pharmacological and toxic actions are closely akin to those of other chaulmoogra preparations.

The toxicity of chaulmoogra preparations is due both to general and local action. According to Read (1924) the general toxicity of chaulmoogra preparations is chiefly due to hydnocarpic acid, which in animals causes hæmolysis of red blood corpuscles and renal irritation, often with hæmoglobinuria and fatty infiltration of the liver. Death in experimental animals is due, not to the formation of multiple emboli in the lungs, but to failure of the respiratory centre.

Dikshit and Row (1931) and Dikshit (1932) found that on intravenous injection, " alepol," a preparation of the sodium salts of the fatty acids of *H. Wightiana* containing those acids which are of low melting-point, causes a reduction in blood pressure ; orally it may cause vomiting, anorexia and some loss in weight ; it has no very definite action on the movements of the small intestine. The irritant effects of " alepol " are decreased when it is prepared in Locke's solution. According to Emerson (1934), the emetic action of the chaulmoogrates is a central one and can be abolished by injections of morphine or atropine. The nauseating effect of " alepol " when given by mouth can often be alleviated by the oral administration of sodium bicarbonate and glucose.

The toxic dose of chaulmoogra preparations for animals has been studied by a number of investigators. Dikshit and Row (1931) found that rabbits tolerate " alepol " in doses of 25 mgm. per kilogram of body weight, while cats tolerate 150 mgm. : with doses of 250 mgm. per kilogram of body weight the animals lose weight.

Sodium chaulmoograte was found by Emerson and Anderson (1934) to be lethal to rats on intravenous injection in doses of from 100 to 125 mgm. per kilogram of body weight. Purified ethyl chaulmoogrates are believed to owe their toxicity to the liberation of sodium chaulmoograte in the tissues. The treatment of ethyl chaulmoograte with 0·5 per cent. iodine has little effect on its toxicity, but the addition of 4 per cent. creosote increases toxicity in proportion to the amount of creosote added. According to Emerson, Anderson and Leake (1933[2]) Na-dichaulmoogryl-β-glycerophosphate (" chaulphosphate "), prepared by Wrenshall in Hawaii, is four to ten times less toxic for animals than " alepol." It is said to liberate chaulmoogric acid in the tissue and to be devoid of hæmolytic action. Six human volunteers received total amounts of 175 mgm. per kilogram of body weight in two weeks without any ill effects and the drug has since been applied to the treatment of leprosy in Brazil (Anderson, Cerqueira, Anderson and Portugal, 1936).

The toxicity for rats of single doses of a number of chaulmoogra preparations is shown in the Table.

The toxic effects of sodium hydnocarpate have been found by Read (1933) to be cumulative, and in time non-inflammatory changes may be produced in the tubular epithelium of the kidneys as well as early necrosis of the liver parenchyma. Frazier (1931), who gave intravenous injections twice weekly for a period of a year, found that these changes could be produced with total doses less than those commonly given to human beings suffering from leprosy. To some extent, however, the body develops a tolerance to chaulmoogra preparations. Anderson, Emerson and Leake (1934) found that in rats repeated doses of the chaulmoogra esters were better tolerated than the water soluble chaulmoogrates. Sodium chaulmoogryl *p*-phenetidine sulphonate and sodium dihydrochaulmoogryl *p*-phenetidine sulphonate caused necrosis at the site of injection when given subcutaneously, and were thus unsuitable for therapeutic trials in animals or man. Ethyl di-N-heptyl acetate, a synthetic preparation introduced by Adams and his co-workers, was found by Lara and Fernández (1932) to compare satisfactorily in the treatment of human leprosy with a

THE TOXICITY FOR RATS OF SINGLE INJECTIONS OF ANTILEPROTIC DRUGS IN AQUEOUS SOLUTION

(Anderson, Emerson and Leake, 1934).

Drug.	Route of Inoculation.	Strength of Solution.	Lethal Range.
(1) Sodium gynocardate .	Intravenous	3 per cent.	300 mgm./kgm.
(2) " Alepol " . . .	,,	3 ,, ,,	100–125 ,,
	Subcutaneous	2 ,, ,,	2·0 gm./kgm.
(3) Sodium chaulmoogryl *p*-phenetidine sulphonate	Intravenous	5 ,, ,,	200–300 mgm./kgm.
	Subcutaneous	6 ,, ,,	500–700 ,,
(4) Sodium dihydro-chaulmoogryl *p*-phenetidine sulphonate	Intravenous	3 ,, ,,	75–100 ,,
	Subcutaneous	2 ,, ,,	400–600 ,,
(5) Ethyl-di-N-heptyl acetate	,,	Undiluted	20 c.c./kgm.
(6) Ethyl esters of chaulmoogra oil	,,	,,	40 ,,

standard ethyl ester preparation of *Hydnocarpus Wightiana* oil. In the treatment of rat leprosy it was found by Anderson, Emerson and Leake (1934) to be inferior to chaulmoogra ethyl esters and to " alepol."

Local Action. In addition to their general action, chaulmoogra preparations produce local effects at the site of injection. The histological effects of localized injections have been specially studied by Nolasco (1930–34). All chaulmoogra preparations injected intradermally cause a cellular infiltration, more especially with large mononuclear cells which become loaded with yellowish oil globules. These loaded cells may persist for at least nine months. Both buffered and non-buffered solutions of sodium hydnocarpate and " alepol " cause rather less cellular infiltration than the iodized ethyl esters, which are therefore possibly rather more efficient. Intracutaneous injections of sodium hydnocarpate and " alepol " tend also to bring about thrombosis of the larger vessels, a result which may limit the value of intracutaneous treatment. Atrophy of the sebaceous glands may also occur as a result of repeated injections of chaulmoogra preparations.

Intradermal injections, as a rule, cause a greater cellular reaction than subcutaneous injections. After intradermal injection chaulmoogra preparations may be detected in the lymph nodes and also within large mononuclear cells in the lungs, much less frequently in the spleen, liver and kidneys. Iodized ethyl esters of hydnocarpus oil injected around nerve trunks produce an acute inflammatory reaction with a fibrinous exudate containing polymorphonuclear leucocytes. The injected material is eventually phagocytosed by large mononuclear cells and carried up and down the limb in the loose perineural tissues.

Considerable attention has been directed to the cause of the irritant action of many chaulmoogra preparations, more especially on intradermal injection. Some samples of chaulmoogric and hydnocarpic acid are more irritant than others, but even the crystalline and oily acid fractions, administered as esters, tend on long exposure to light to become highly irritant. This irritant power is associated with the development of cloudiness, a fall in specific rotation and a change in *p*H to the acid side. Paget, Trevan and Attwood (1934) found that the irritant action was associated with the development of syrupy acids and sticky varnishes which contain large amounts of lactonic acid. Cole (1935) believes that pure hydnocarpic acid decomposes more rapidly than pure chaulmoogric acid. Decomposition is more rapid under tropical than under more temperate conditions. Cole (1929) states that treatment of the ethyl esters of *Hydnocarpus Wightiana* oil with iodine greatly reduces the irritant action.

From time to time claims have been made for the chemotherapeutic potency of animal and vegetable oils other than those obtained from the Flacourtiaceæ.

Oleum Morrhuæ was at one time thought to be of value in leprosy, but apart from its action in improving the general health it seems to have little specific action on *Mycobacterium lepræ*. Rao (1935), for instance, found that in cases with symmetrical lesions, intradermal injection of iodized ethyl hydnocarpate was much more effective than a mixture of ethyl morrhuate and ethyl oleate, though the latter was more irritating. Jdan-Pushkin and Kuznetzov (1933) claim to have obtained successful results in leprosy with oil containing unsaturated fatty acids obtained from

Perilla ocymides, a labiate plant growing in India, China, Japan and the Far East of the Soviet Union. These results lack confirmation. The oil obtained from the seeds of *Pongamia glabra*, a leguminous tree common in India, has also a local reputation as a remedy for skin diseases, but Rao (1934) has found it useless in leprosy.

References

Anderson, H. H., Cerqueira, P., Anderson, J. van D., and Portugal, H., 1936, Clinico-pathologic studies of leprosy in Brazil. *Amer. J. trop. Med.*, XVI., 689.

Anderson, H. H., Emerson, G., and Leake, C. D., 1934, A pharmacological evaluation of certain antileprotic drugs. *Int. J. Leprosy*, II., 39.

Cole, H. I., 1929, Reduction of irritation by iodized ethyl esters of *Hydnocarpus Wightiana* oil. *Philipp. J. Sci.*, XL., 503.

Cole, H. I., 1935, Note on the decomposition products in Chaulmoogra oils. *Int. J. Leprosy*, III., 81.

Dikshit, A. B., and Row, R. S. T. M., 1931, A preliminary note on the actions and uses of " alepol." *Indian med. Gaz.*, LXVI., 317.

Dikshit, B. B., 1932, Alepol in leprosy. *Indian med. Gaz.*, LXVII., 7.

Emerson, G. A., 1934, Mechanism of the emetic action of the chaulmoogrates. *Proc. Soc. exp. Biol. N.Y.*, XXXII., 238.

Emerson, G. A., and Anderson, H. H., 1934, Acute toxicity of ethyl chaulmoograte. *Proc. Soc. exp. Biol. N.Y.*, XXXII., 289.

Emerson, G., Anderson, H. H., and Leake, C. D., 1933 [1], Lipolytic activity of rat lepromata during treatment with various anti-leprotics. *Proc. Soc. exp. Biol. N.Y.*, XXXI., 18.

Emerson, G., Anderson, H. H., and Leake, C. D., 1933 [2], Comparative biological activity of seven new water-soluble chaulmoogric acid derivatives. *Proc. Soc. exp. Biol. N.Y.*, XXXI., 274.

Frazier, C. N., 1931, Experimental production of nephrosis-like lesions by sodium hydnocarpate. *Proc. Soc. exp. Biol. N.Y.*, XXIX., 44.

Jdan-Pushkin, M. N., and Kuznetzov, V. N., 1933, Fatty vegetable oils in the treatment of leprosy. *Urol. cutan. Rev.*, XXXVII., 851.

Lara, C. B., and Fernández, G., 1932, Clinical trial of ethyl di-N. heptyl acetate in leprosy. *Trans. 8th Congr. Far East. Ass. trop. Med.*, Siam, 1930, II., 575.

Nolasco, J. O., 1930 [1], Histopathology of leprosy under local infiltration. II. The nature of the yellowish globules in treated lesions. *J. P. I. med. Ass.*, X., 273.

Nolasco, J. O., 1930 [2], Local effects of infiltration with buffered and non-buffered solutions of sodium hydnocarpate and alepol. *J. P. I. med. Ass.*, X., 277.

Nolasco, J. O., 1931, Local effects of infiltration with iodized ethyl esters of *Hydnocarpus Wightiana* oil in non-lepers. *J. P. I. med. Ass.*, XI., 219.

Nolasco, J. O., 1932, Lymphatic absorption of oily antileprotic drugs given intradermally and subcutaneously : a demonstration. *J. P. I. med. Ass.*, XII., 147.

Nolasco, J. O., 1934 [1], Local effects of injection of iodized Wightiana ethyl esters and Wightiana oil round nerve trunks. *J. P. I. med. Ass.*, XLV., 421.

NOLASCO, J. O., 1934[2], Histologic studies on the plancha or infiltration method of leprosy treatment. *Int. J. Leprosy*, II., 159.

PAGET, H., TREVAN, J. W., and ATTWOOD, A. M. P., 1934, The irritant constituent of anti-leprotic oils. *Int. J. Leprosy*, II., 149.

RAO, G. R., 1932, Studies on serum proteins in leprosy with special reference to Hydnocarpus treatment. *Indian J. med. Res.*, XIX., 993.

RAO, G. R., 1934, Ethyl esters of " Pongamia glabra " in the treatment of leprosy. *Leprosy Rev.*, V., 127.

RAO, G. R., 1935, A comparative study of the relative efficacy of ethyl hydnocarpate and ethyl morrhuate in leprosy. *Leprosy Rev.*, VI., 120.

READ, B. E., 1924, The toxicity of Chaulmoogra oil (Oleum hydnocarpi). *J. Pharmacol.*, XXIV., 221.

READ, B. E., 1933, The toxicity of sodium hydnocarpate. *Int. J. Leprosy*, I., 293.

THE TREATMENT OF LEPROSY BY CHAULMOOGRA AND ALLIED OILS

The following chaulmoogra preparations (*cf.* Walker Tomb, 1933) have been employed in the treatment of human leprosy :—

(1) *Hydnocarpus Wightiana* or *H. anthelmintica* oil.

(2) Ethyl esters of the fatty acids of chaulmoogra, hydnocarpus and sapucainha oils.

(3) Iodized ethyl esters.

(4) Sodium salts of the fatty acids of *H. Wightiana.* " Alepol " consists of the sodium salts of a selected fraction of the low melting acids of *H. Wightiana* oil.

(5) Sodium dichaulmoogryl-β-glycerophosphate (" chaulphosphate ").

The methods of administration of these preparations are oral, intramuscular, intradermal, intravenous, subcutaneous and intranasal (*cf.* Samson, Lara and Cruz, 1930, and Dimitry, 1931).

(1) *Hydnocarpus Wightiana* or in Indo-China *H. anthelmintica* oil (*cf.* Souchard and Ramijean, 1933) is sometimes given by mouth, but is more commonly administered by other routes ; intravenous injections are, however, usually avoided, though Labernadie (1934) reports an entire absence of toxic reactions after intravenous injections of 1 c.c. of exactly neutralized oil. Oil expressed cold from fresh ripe seeds is only very slightly irritating when injected, but as Lowe and De (1937) point out, it should be entirely free from dust and particulate matter.

Deterioration due to oxidation is also important (Paget, Trevan and Attwood, 1934).

(2) The ethyl esters of chaulmoogric or hydnocarpic acids are of special importance from the ease with which they can be administered intradermally (*cf.* Muir, 1931). The factors involved in the esterification of chaulmoogra oils are discussed very fully by Cole and Cardoso (1936), who also describe experiments on the influence of sulphuric acid, acting as a catalyst, on the rate of the reaction. Total ethyl esters were first used by Engel Bey (1910).

The ethyl esters of hydnocarpus oil should be soluble in not less than six volumes of cold alcohol (90 per cent.). Sp. G. (15·5°/15·5°) 0·905 to 0·910 ; optical rotation not less than + 45° ; refractive index at 20°, 1·458 to 1·462 ; acid value not greater than 1·0 ; saponification value 190 to 196 ; iodine value 88 to 94.

A number of simple and complex esters of the mixed acids from chaulmoogric and hydnocarpic oils or of pure chaulmoogric or pure hydnocarpic acid has been made by Perkins (1924), Santiago and West (1928), De Santos and West (1929) and Santillan and West (1929). So far none of these has proved to have any clinical advantage over the raw oils, ethyl esters or sodium salts.

A number of chaulmoogra esters of amino-alcohols combined with cholesterol has been prepared by Baranger ; these have been used for intravenous injections by Flandin, Baranger and Ragu (1936). They can be sterilized at 115° C. and are non-hæmolytic, giving rise to fine ultramicroscopic stable suspensions. Doses of 6 to 7 c.c. have been given intravenously. No reactions occurred in twelve patients with leprosy treated in Paris, but in early or acute cases the evolution of the disease was arrested and in more advanced cases the leprosy bacilli rapidly disappeared and the condition became stabilized. Oto, Sato and Masuzawa (1935) have prepared stable emulsions of chaulmoogra ethyl esters in which the particles are not larger than 1μ in diameter. These emulsions, when injected intravenously in single doses of from 2·5 to 5 c.c., are said to have caused no unpleasant symptoms.

(3) Iodized ethyl esters. It has been found (Perkins, 1922)

that the addition of 0·5 per cent. of iodine to the ethyl esters renders them less irritating without affecting their efficiency. The esters are heated rapidly to 140° C., the iodine is added and the dark solution maintained at 150° C. for twenty minutes, filtered and stored in well-closed bottles before use. Keil (1935) advocates the addition of 10 per cent. instead of only 0·5 per cent. iodine. Although the majority of observers believe that the therapeutic efficiency of the iodized ethyl esters is due entirely to their chaulmoogra content, Lara and Samson (1932) have brought forward evidence that olive oil esters containing 0·5 per cent. iodine and 2 per cent. benzocaine also possess some therapeutic activity.

Phenylethyl esters (" eulykol ") may possibly be of value in patients who have found the ordinary or the iodized ethyl esters too irritating.

(4) The sodium salts or soaps of the total fatty acids of *H. Wightiana* or, less commonly, *H. anthelmintica* have been employed, especially by French workers such as Souchard and Ramijean (1933), who have given by mouth as many as twenty pills a day, each of 0·3 cgm., for as long as five months. Old nodular cases especially were improved. British workers for the most part prefer " alepol " to the sodium salts of the total fatty acids, since it can be injected not only intramuscularly or subcutaneously, but intravenously also, provided precautions are taken to draw blood from the vein into the syringe and mix it with the material to be injected. For intravenous injection Dikshit (1932) recommends a 1 or 2 per cent. solution of " alepol " made up in Locke's solution containing no glucose.

(5) Sodium dichaulmoogryl-β-glycerophosphate " chaulphosphate " was introduced because it appeared to cause much less hæmolysis than the sodium salts of the acids themselves. " Chaulphosphate " is reported by Hurwitz and Anderson (1936) and Anderson, Cerqueira, Anderson and Portugal (1936) to have been used clinically in man with excellent results : further comparative observations of its efficiency are required. Recently Wagner-Jauregg and Arnold (1937) have suggested that this substance may have antigenic properties.

The synthesis of *dl*-chaulmoogric acid has been claimed by Perkins and Cruz (1927 [1 & 2]), while Stanley and Adams (1929) believe that they have synthesized from hydnocarpic acid a substance which agrees in every respect with the naturally occurring chaulmoogric acid. Adams (*cf.* Stanley and Adams, 1932) has also synthesized *cyclo*-pentylalkyl acetic acids which, *in vitro* at any rate, show an inhibitory action on the growth of certain acid-fast bacilli.

Although it is now generally agreed that treatment by chaulmoogra, so long as it is not pushed so far as to produce severe leprotic reactions, has a very definite curative action in leprosy, it is difficult, if not impossible, to assess the values of particular chaulmoogra preparations. This is due both to the variations that occur in the severity of leprosy in different countries and to the differences of environment to which lepers are subjected, for without adequate diet, good surroundings and the treatment of intercurrent disease, the chemotherapeutic activity of chaulmoogra will be inhibited or impaired. In what this chemotherapeutic activity consists is still unknown, for though no evidence of a direct bactericidal action is forthcoming, owing to the impossibility of cultivating *Mycobacterium lepræ*, evidence is equally lacking of any indirect action on the tissues, though possibly the increase in large mononuclear cells which follows an injection of chaulmoogra oil may increase the rate of destruction of the bacilli. Irritation of the tissues is not the primary cause of the action of the chaulmoogra compounds, for Lara and Lagrosa (1932) have pointed out that the injection of ethyl stearate or ethyl oleate is more irritating than that of chaulmoogra ethyl esters, but is therapeutically useless. The observations of Emerson, Anderson and Leake (1934) showed that chaulmoogra preparations do not act therapeutically through an indirect stimulation of the lipolytic activity of the tissues. The relative value of chaulmoogra preparations must for the present be assessed on their lack of toxicity, ease of administration, and cost, rather than on their specific action on the bacilli of leprosy.

References

Anderson, H. H., Cerqueira, P., Anderson, J. van D., and Portugal, H., 1936, Clinico-pathologic studies of leprosy in Brazil. *Amer. J. trop. Med.*, XVI., 689.

Cole, H. I., and Cardoso, H., 1936, Purification and esterification of chaulmoogra oil. *Int. J. Leprosy*, IV., 455.

Dikshit, B. B., 1932, Alepol in leprosy. *Indian med. Gaz.*, LXVII., 7.

Dimitry, T. J., 1931, The treatment of the nasal passages and eyes with chaulmoogra oil in leprosy. *Amer. J. trop. Med.*, XI., 65.

Emerson, G. A., Anderson, H. H., and Leake, C. D., 1934, A pharmacological comparison of Na-hydnocarpate (" Alepol ") and Na-dichaulmoogryl-β-glycerophosphate (" Chaulphosphate "). *Arch. int. Pharmacodyn.*, XLVIII., 247.

Engel-Bey, 1910, Zur Behandlung der Lepra mit Antileprol. *Lepra.*, XI., 368.

Flandin, C., Baranger, P., and Ragu, J., 1936, Essai de traitement de la lèpre par un complex nouveau de chaulmoogra et de cholésterol, permettant l'injection intraveineuse à haute dose de dérivés chaulmoogriques. *C. R. Acad. Sci.*, CCIII., 502.

Hurwitz, E., and Anderson, H. H., 1936, Leprosy in Panama. First thirty years of segregation. *Amer. J. trop. Med.*, XVI., 353.

Keil, E., 1935, Zur Behandlung der Lepra mit Jod-Antileprol. *Arch. Schiffs- u. Tropenhyg.*, XXXIX., 188.

Labernadie, V., 1934, Essais de traitement de lépreux par des injections intraveineuses d'huile de chaulmoogra (résultats obtenus après six mois de traitement). *Ann. Méd. Pharm. colon.*, XXXII., 328.

Lara, C. B., and Lagrosa, M., 1932, Observations bearing on the question of whether or not the chaulmoogra-group drugs have any special action in leprosy. I. Changes in leprotic skin lesions following intradermal injections of various oily preparations. *J. P. I. med. Ass.*, XII., 599.

Lara, C. B., and Samson, J. G., 1932, Role of iodine in iodized oil derivatives used as antileprotic drugs : trial of plain and iodized olive oil ethyl esters. *J. P. I. med. Ass.*, XII., 485.

Lowe, J., and De, N. K., 1937, Tests on the suitability of hydnocarpus oil for injection. *Leprosy in India*, IX., 49.

Muir, E., 1931, The treatment of leprosy. *Trans. R. Soc. trop. Med. Hyg.*, XXV., 87.

Oto, M., Sato, S., and Masuzawa, T., 1935, A chaulmoogra preparation for intravenous use and its therapeutic effect. *Int. J. Leprosy*, III., 153.

Paget, H., Trevan, J. W., and Attwood, A. M. P., 1934, The irritant constituent of anti-leprotic oils. *Int. J. Leprosy*, II., 149.

Perkins, G. A., 1922, Manufacture of certain drugs for the treatment of leprosy. *Philipp. J. Sci.*, XXI., 1.

Perkins, G. A., 1924, Chaulmoogra ethyl, propyl, butyl and amyl esters. *Philipp. J. Sci.*, XXIV., 621.

Perkins, G. A., and Cruz, A. O., 1927[1], Synthesis of compounds similar to chaulmoogric acid. I. *J. Amer. chem. Soc.*, XLIX., 517.

Perkins, G. A., and Cruz, A. O., 1927[2], Synthesis of compounds similar to chaulmoogric acid. II. *J. Amer. chem. Soc.*, XLIX., 1070.

Samson, J. G., Lara, C. B., and Cruz, M. C., 1930, Treatment of leprous lesions of the nasal mucosa. *J. P. I. med. Ass.*, X., 291.

Santiago, S., and West, A. P., 1928, Chaulmoogryl derivatives of lactates and salicylates. *Philipp. J. Sci.*, XXXV., 405.

SANTILLAN, P., and WEST, A. P., 1929, Chaulmoogryl brom and chlor phenols. *Philipp. J. Sci.*, XL., 493.

SANTOS, I. DE, and WEST, A. P., 1929, Chaulmoogryl substituted phenols and chaulmoogryl hydroxy ethyl benzoate. *Philipp. J. Sci.*, XXXVIII., 293.

STANLEY, W. M., and ADAMS, R., 1929, The synthesis of chaulmoogric acid from hydnocarpic acid. *J. Amer. chem. Soc.*, LI., 1515.

STANLEY, W. M., and ADAMS, R., 1932, Bacteriological action of certain synthetic organic acids towards *Mycobacterium lepræ* and other acid-fast bacilli. *J. Pharm. exp. Ther.*, XLV., 121.

SOUCHARD, L., and RAMIJEAN, 1933, Contribution à l'étude du traitement de la lèpre par les savons de " Krabao." *Arch. Inst. Pasteur Indo-Chine*, No. 18, 187.

WAGNER-JAUREGG, T., and ARNOLD, H., 1937, Darstellung der α,γ-Dichaulmoogroyl-glycerin-β-phosphorsaüre. *Ber. dtsch. chem. Ges.*, LXX., 1459.

WALKER TOMB, J., 1933, Chaulmoogra oil and its derivatives in leprosy. *J. trop. Med.*, XXXVI., 170, 186 and 201.

ANILINE DYES

When aniline dyes are injected intravenously into animals they are taken up by the macrophages of the reticulo-endothelial system and by connective tissue cells. Vital staining with acid dyes results, as Ludford (1931) has pointed out, in the production of new formations within the cells, while basic dyes stain pre-existing granules and vacuoles in the cytoplasm. The action of the dye thus depends less on its chemical composition than on its physical properties. The possibility that certain aniline dyes might have a favourable action on leprosy first suggested itself to Ryrie (1933) when he observed the selective concentration of methylene blue in leprotic nodules following intravenous injection. The therapeutic action of a number of dyes was, therefore, tested in lepers. Little or no result was obtained from the intravenous injection of methylene blue, crystal violet, picric acid, malachite green, rhodamine, Congo red, iodine green, acriflavine, methyl orange, toluidine blue, acid fuchsin, basic fuchsin, indigo carmine, chrysoidine, auramine and Giemsa's stain.

Some improvement was, however, noted after the intravenous injection of trypan blue (4 per cent. solution), brilliant green (1 per cent.) and fluorescein (1 or 2 per cent.). Eosin also appeared to produce clinical improvement. In lepra reactions fluorescein gave good results in the majority of cases (Ryrie, 1934). The essential constituent in fluorescein appeared to be phthalic acid, for resorcin, the other component of fluorescein, had no effect. Unfortunately the beneficial effects of fluorescein are of a temporary nature, although it may be of value when administered between courses of chaulmoogra. Fluorescein was found by Fernández and Schujman (1935) to be of some value in the treatment of leprous patients with iritis and other acute ocular symptoms, but valueless in other forms of leprosy. Eosin was also without any curative action.

Brilliant green has also received further investigation, for it forms one of the constituents of "Bonney's blue." This preparation consists of brilliant green 0·5 gm., crystal violet 0·5 gm., absolute alcohol 25 c.c., distilled water to 2,500 c.c. This solution was either sprayed on to ulcerative lesions or injected intradermally by Ryles (1933), who claimed very rapid improvement. Leggate (1934) obtained similar results, injections being given not only intradermally, but intravenously in doses of 3 to 12 c.c. once a week. Sorley (1934) claimed improvement after intravenous injections of 1 per cent. brilliant green in doses of 3 to 5 c.c. twice weekly. Toxic reactions such as precordial pain, dyspnœa, vomiting, albuminuria and jaundice were not uncommon.

Trypan blue has also been tested by Muir and Chatterji (1934) in leprous eye lesions. Subconjunctival injections of a 0·1 per cent. solution in normal saline were given, as well as intravenous injections of a 1 per cent. solution in doses of 3 to 20 c.c. once or twice a week. In eleven patients pain was relieved and vision improved. Rao (1935) has not been able to confirm these results, for he was unable to detect any appreciable effects on the course of the disease or on the causative organisms.

Anderson, Emerson and Fisher (1934), in addition, pointed out that, in the doses recommended for human beings, trypan blue, brilliant green and crystal violet were all toxic. Trypan blue had a depressant action on the central nervous system and when injected intravenously varied greatly in its toxicity for animals. Brilliant green and gentian violet seemed to be injurious to the liver. In leprous rats gentian violet and brilliant green are too toxic for continued administration, though trypan blue appears to be better tolerated and to have a higher leprocidal activity. The toxicity of trypan blue is fully discussed by Steyn (1935) and by Emerson and Anderson (1934), who have shown that its action is cumulative. Fluorescein, eosin, erythrosin and methylene green were lethal for rabbits injected intravenously in doses of 300, 350, 200 and 150 mgm. per kilogram of body weight, respectively, and in doses of 600, 500, 300 and 125 mgm. per kilogram intraperitoneally in rats. Orally in rats these dyes were tolerated in doses of 1·0 gm. per kilogram of body weight, with the exception of methylene green, which killed two out of five rats when given in a dose of 500 mgm. per kilogram of body weight.

Although Ryrie (1933) did not cause any deaths he noted that reactions occasionally followed the intravenous injection of drugs in man. Cardiac pain, epigastric palpitation, gastric and rectal irritation, feeble pulse and signs of shock usually lasted but a short time. Exceptionally coma and temporary failure of the heart and respiration necessitated active measures of resuscitation.

Ryrie (1933) failed to note any improvement after injections of methylene blue. French workers, however, claim to have obtained remarkable improvement with this compound. Montel (1934 [1 & 2]), who initiated this form of treatment, gave intravenous injections of a 1 per cent. neutral solution of methylene blue intravenously to 172 lepers over a period of nine months. The solutions were heated for three consecutive days for one hour at 80° C. The first dose of 5 c.c. was increased to the limits of tolerance, usually between 25 and 35 c.c., injections being given three times a week.

Both cutaneous and nerve cases are said to have improved rapidly. These results have given rise to an extensive and somewhat acrimonious literature, for while Fréville (1934 [1 & 2]), Bigot and Le-van-Trien (1934), Afanador (1934) and Nicolas (1935) claim that methylene blue produces rapid improvement, many others, Raynaud, Alcay and Huguenin (1935), Dubois, Westerlinck and Degotte (1935), Delanoe (1935), Dubois (1935), Lépine and Markianos (1935), failed signally to produce any striking amelioration. Montel (1936) attributes these failures to insufficient dosage and believes that doses of less than 25 c.c. may cause reactivation of the lesions: the minimum effective dose is said to be 5 mgm. per kilogram of body weight, but this should be rapidly increased to 8 or even 10 mgm. per kilogram of body weight. It is claimed that of 351 patients who received treatment for more than three months :—

10·26 per cent. became bacteriologically negative ;
34·76 per cent. were notably improved ;
45·87 per cent. were improved ;
6·55 per cent. remained stationary ;
1·42 per cent. became worse ;
1·14 per cent. died.

The exact value of these figures, in so far as methylene blue treatment is concerned, is doubtful, for in many the injections of methylene blue were associated with the administration of chaulmoogra and no control figures for treatment with chaulmoogra alone were provided. Insufficient dosage does not apply to the results obtained by Bertaccini (1936), who treated twenty patients of the nodular type, two of the nerve type and three of the mixed forms. Gradually increasing doses of from 5 to 40 c.c. of a 1 per cent. solution were given two to three times a week, and, in addition, injections of 1 c.c. were made either into the neighbourhood of the nodules or directly into the nodules. Direct intranodular injections often lead to disintegration and ulceration and in a few patients the nodules softened and diminished in size, but in a majority, apart from the selective staining, no visible changes were noted. Bacilli did not disappear from the nodules nor from the nasal mucous membrane : neither anæsthesia nor articular pains were affected and the general health was not improved. For the present, therefore, the specific action of methylene blue must be regarded as non-proven, though the dye undoubtedly has a selective action on infiltrative lesions and possibly a disintegrative action on the leprosy bacillus (Marchoux and Chorine, 1935 ; Lépine and Markianos, 1935). In addition methylene blue may produce toxic reactions and is contra-indicated in patients with renal or hepatic damage. Bertaccini (1936) found that vomiting, diarrhœa, dyspnœa, cyanosis, giddiness and tachycardia followed intravenous injections of the drug. Fréville (1934 [2]) noted acute cardiac failure which was not prevented by adding adrenalin to the use of solutions prepared without heat from previously sterilized materials. Montel (1935) found that injections of methylene blue predisposed to the development of acne or furunculosis over the lesions. This, however, was generally regarded as a favourable sign, though bacterial infection might necessitate cessation of treatment.

The use of methylene blue obviously requires further investigation ; its administration by mouth is said by Berny (1936) to relieve nerve pains with remarkable rapidity.

Certain other dyes have been used to a small extent in the treatment of leprosy. Léger (1930) claimed to have found trypaflavine of value, but Schwetz (1933) failed to obtain any improvement. Montel, Montel and Nhuan (1936) found that phenol sulphophthalein localized in the leprous nodules and produced some subjective and objective improvement. One patient developed purpura. Grimes, Cluzet and Minec (1935) used gentian violet in Madagascar in doses of 3 mgm. per kilogram of body weight. Intravenous injections of a 10 per cent. solution were given twice a week.

It cannot so far be said that the aniline dyes have provided a chemotherapeutic agent that can replace chaulmoogra and its derivatives, nevertheless, the power possessed by certain dyes of staining lepromata selectively suggests that further research may possibly reveal some dye with high leprocidal action.

References

AFANADOR, A., 1934, Traitement de la lèpre par les injections intraveineuses de bleu de méthylène. *Bull. Soc. Path. exot.*, **XXVII.**, 805.

ANDERSON, H. H., EMERSON, G. A., and FISHER, B. H., 1934, Acute toxicity of trypan blue, gentian violet and brilliant green. *Proc. Soc. exp. Biol. N.Y.*, **XXXI.**, 825.

BERNY, P., 1936, Action du bleu de méthylène administré par voie buccale sur les douleurs des lépreux. *Bull. Soc. Path. exot.*, **XXIX.**, 30.

BERTACCINI, G., 1936, Sulla cura della lebbra col bleu di metilene. *G. ital. Derm. Sif.*, **LXXVII.**, 561.

BIGOT, A., and LE-VAN-TRIEN, 1934, Trois cas de lèpre traités par le méthode de Montel, au bleu de méthylène. *Bull. Soc. méd.-chir. Indo-Chine*, **XII.**, 615.

DELANOË, MME E., 1935, Le bleu de méthylène compris dans le traitement mixte de la lépre. *Bull. Soc. Path. exot.*, **XXVIII.**, 348.

DUBOIS, A., 1935, Essais thérapeutiques dans la lèpre. Le bleu de méthylène. *Bull. Soc. Path. exot.*, **XXVIII.**, 63.

DUBOIS, A., WESTERLINCK, H., and DEGOTTE, J., 1935, Essais thérapeutiques dans la lèpre. Le bleu de méthylène. *Bull. Soc. Path. exot.*, **XXVIII.**, 63.

EMERSON, G. A., and ANDERSON, H. H., 1934, Toxicity of certain proposed antileprosy dyes : fluorescein, eosin, erythrosin and others. *Int. J. Leprosy*, **II.**, 257.

FERNÁNDEZ, J. M. M., and SCHUJMAN, S., 1935, On the use of fluorescein in the treatment of leprosy. *Leprosy Rev.*, **VI.**, 182.

FRÉVILLE, L H. F., 1934 [1], Note relative à quelques essais d'injections intraveineuses de solution iodo-iodurée dans la lèpre. Premiers résultats. *Bull. Soc. méd.-chir. Indo-Chine*, **XII.**, 756.

FRÉVILLE, L. H. F., 1934 [2], Recherches expérimentales sur les réactions produites par les injections intraveineuses de bleu de méthylène dans la lèpre. *Bull. Soc. méd.-chir. Indo-Chine*, **XII.**, 615.

GRIMES, C., CLUZET, and MINEC, 1935, Note préliminaire sur un essai de traitement de la lèpre à Madagascar par le violet de gentiane. *Bull. Soc. Path. exot.*, XXVIII., 415.

LÉGER, M., 1930, Association au chaulmoogra de divers autres médicaments pour le traitement de la lèpre. La trypaflavine. *Bull. Soc. Path. exot.*, XXIII., 1009.

LEGGATE, J., 1934, Bonney's blue solution in the treatment of leprosy. *Leprosy Rev.*, V., 161.

LÉPINE, P., and MARKIANOS, J., 1935, Action directe du bleu de méthylène sur le bacille de Hansen dans l'organisme humain. *C. R. Soc. Biol.*, CXVIII., 9.

LUDFORD, R. J., 1931, The vital staining of normal and malignant cells: a comparison of vital staining of parenchyma cells and liver with acid and basic dyes. *Proc. Roy. Soc. B.*, CVIII., 270.

MARCHOUX, E., and CHORINE, V., 1935, Action du bleu de méthylène sur les lépromes "in vivo." *Bull. Acad. Méd.*, CXIII., 10.

MONTEL, R., 1934 [1], Un nouveau traitement de la lèpre: note préliminaire. *Bull. Soc. Path. exot.*, XXVII., 220.

MONTEL, R., 1934 [2], Traitement de la lèpre par le bleu de méthylène en injections intraveineuses. *Bull. Acad. Méd.*, CXII., 208.

MONTEL, R., 1935, Poussées de lépromes furonculoides au cours du traitement par le bleu de méthylène. *Bull. Soc. méd.-chir. Indo-Chine*, XIII., 9.

MONTEL, R., 1936, Conférence sur le traitement de la lèpre par le bleu de méthylène. *Bull. Soc. Path. exot.*, XXIX., 361.

MONTEL, R., MONTEL, G., and NGUYEN NGOC NHUAN, 1936, Essais de traitement de la lèpre par la phénol-sulfone-phthaléine. *Bull. Soc. Path. exot.*, XXIX., 1064.

MUIR, E., and CHATTERJI, S. P., 1934, Trypan blue in the treatment of leprous eye lesions. *Leprosy Rev.*, V., 192.

NICOLAS, C., 1935, Bleu de méthylène et lèpre. *Bull. Soc. Path. exot.*, XXVIII., 10.

RAO, G. R., 1935, Studies on the therapeutic efficacy of certain dyes in leprosy. *Leprosy Rev.*, VI., 4.

RAYNAUD, ALCAY, and HUGUENIN, 1935, A propos de deux cas de lèpre. *Soc. méd. d'Alger.* (January 18th).

RYLES, C. S., 1933, Brilliant green and crystal violet in the treatment of leprosy. *Leprosy Rev.*, IV., 99.

RYRIE, G. A., 1933, A preliminary report on the action of certain dyes in leprosy. *Trans. R. Soc. trop. Med. Hyg.*, XXVII., 85.

RYRIE, G. A., 1934 [1], The use of fluorescein in lepra reaction. *Leprosy Rev.*, V., 12.

RYRIE, G. A., 1934 [2], On the use of fluorescein and phthalic acid in leprosy. *Int. J. Leprosy*, II., 139.

SCHWETZ, J., 1933, A propos du traitement de la lèpre par la trypaflavine. *Ann. Soc. belge. Méd. trop.*, XIII., 43.

SORLEY, J. T., 1934, The use of brilliant green intravenously in the treatment of leprosy. *West. Afric. med. J.*, VIII. (No. 2), 13.

STEYN, D. G., 1935, The toxicity of trypan blue. *Onderstepoort J. vet. Sci.*, V., 467.

METALLIC PREPARATIONS IN THE TREATMENT OF LEPROSY

Various metals have from time to time been recommended in the treatment of leprosy. Gold, in the form of " solganal-B," has, it is claimed, a definite therapeutic effect, especially in eye cases. Muir (1932), however, found that so far from improving leprosy, intramuscular injections of " solganal-B " lowered the general resistance and, in some cases, set up intraocular inflammation. Dubois and Ury (1933) also failed to obtain improvement after the use of " solganal," " solganal-B," " chrysalbin," " allochrysine " and " lopion." " Sanocrysin " alone was without effect, while with chaulmoogra oil and " sanocrysin " results were no better than with chaulmoogra alone. Favourable results have, however, been claimed by Alfred (1933) and Cochrane (1933), while Paldrock (1935) has combined the administration of gold salts with local applications of carbon dioxide snow. Cochrane and Paul Raj (1937) have used " solganal-B oleosum " and obtained slight amelioration in mildly reactive cases, provided the dose did not exceed 0·1 gm. Ueno (1936) claims to have produced considerable improvement in twenty-two patients treated with a gold soap solution in chaulmoogra oil.

Copper and antimony preparations have also been tested, but in stationary cases have no effect. Leprosy reactions, however, are frequently relieved by the intravenous injection of potassium antimonyl tartrate in doses of 1 to 5 c.c. of a 1 per cent. solution every two days.

While mercury has no action on the lepromata as such, the intravenous injection of " mercurochrome " in doses of from 3 to 10 c.c. is recommended by Muir and Chatterji (1933) in septic conditions and in lepra reactions. An organic mercurial, 2-myristoxymercuri-3-hydroxybenzaldehyde (" avenyl "), prepared by Henry and Sharp (1922) and dissolved in hydnocarpus oil, has proved of value in the treatment of syphilitic infections in lepers.

Many other preparations such as " cysteine " (Montel, Montel and Le Van Phung, 1936) have been used in the treatment of leprosy, but their exact value must await further and more detailed observations.

References

ALFRED, E. S. R., 1933, Solganal in the treatment of leprosy. *Leprosy Rev.*, IV., 16.

COCHRANE, R. G., 1933, The modern treatment of leprosy. *Leprosy Rev.*, IV., 69.

COCHRANE, R. G., and PAUL RAJ, M., 1937, Solganal-B oleosum in the treatment of leprosy. *Leprosy in India*, IX., 75.

DUBOIS, A., and URY, J., 1933, Note sur le traitement de la lèpre par les sels d'or. *Ann. Soc. belge. Méd. trop.*, XIII., 5.

HENRY, T. A., and SHARP, T. M., 1922, Mercury compounds of hydroxybenzaldehydes. *J. chem. Soc.*, CXXI., 1055.

MONTEL, R., 1936, Conférence sur le traitement de la lèpre par le bleu de méthylène. *Bull. Soc. Path. exot.*, XXIX., 361.

MONTEL, R., MONTEL, G., and LE VAN PHUNG, 1936, Essais de traitement de la lèpre par la cystéine en injections intraveineuses. *Bull. Soc. Path. exot.*, XXIX., 1061.

MUIR, E., 1932, Solganal-B in the treatment of leprosy. *Leprosy in India*, IV., 6.

MUIR, E., and CHATTERJI, S. P., 1933, Further notes on mercurochrome. *Leprosy in India*, V., 8.

PALDROCK, A., 1935, Durch spezifische Behandlung von Lepra geheilt. *Arch. Schiffs- u. Tropenhyg.*, XXXIX., 23.

UENO, S., 1936, On the preparation of a gold soap solution in chaulmoogra oil and its therapeutic effect on lepers. *J. Soc. chem. Ind. Japan.*, XXXIX., Supp., 151.

CHAPTER IX

THE CHEMOTHERAPY OF ACUTE BACTERIAL INFECTIONS

Although some success had been obtained by Morgenroth and Levy (1911) in the treatment of pneumococcal infections in mice with ethyl dihydrocupreine, up to a short time ago protection against streptococci had been entirely unsuccessful. In the course of extensive investigations carried out by the I. G. Farbenindustrie, Elberfeld, on the bactericidal action of azo dyes, it was noted that the presence of a sulphonamide group, while decreasing the efficiency of the dyes as antiseptics, caused them to protect mice infected with hæmolytic streptococci (Hörlein, 1937). The first azo dyes containing sulphonamide and substituted sulphonamide groups were prepared by Hörlein, Dressel and Kothe (1909 and 1910). One of these dyes, sulphamidochrysoidin (prontosil), the hydrochloride of 4-sulphamido-2′ : 4′-diaminoazobenzene, had been synthesised by Mietzsch and Klarer in 1932 and had been tested clinically to a small extent by physicians in the Rhineland (*cf.* Foerster, 1933, and Grütz, 1934). The first full account, however, of the action of this compound was given by Domagk (1935 [1]), who found that a single dose of prontosil varying from 0·02 to 10 mgm. given to a mouse an hour and a half after infection was effective against a lethal dose of at least one strain of hæmolytic streptococci inoculated into the peritoneum. Scrapings of peritoneum taken forty-eight hours after infection were free from cocci in treated mice, whereas in controls vast numbers of organisms were present. In a later paper Domagk (1935 [2]) stated that the administration of 0·2 to 1 mgm., that is one-tenth to one-fiftieth of the tolerated dose, for three to five days after infection protected most of the mice, while certain less acute infections could be controlled by 1/100 to 1/500 of the tolerated dose. Levaditi and Vaisman (1935^1) quickly confirmed these results, although they could not protect every mouse: cocci were recovered from the spleens of treated mice up to the eighth day after infection, but if the animals survived for a longer period the spleens became sterile. Nitti and Bovet (1935) observed that strains of streptococci of low virulence were less affected by the drug. The most important finding, however, was that of Tréfouël, Tréfouël, Nitti and Bovet (1935), who noticed, following the observation of Heildelberger and Jacobs (1919) on the bactericidal action *in vitro* of sulphamidobenzeneazohydrocupreines, that azo-compounds which did not contain a sulphonamide group attached to one of the two benzene nuclei were inactive, whereas the substituent groups in the other nucleus could be varied considerably without influencing the protective power. This led them to the conclusion that the animal was able to break down the compounds with the formation of *p*-aminobenzenesulphonamide, a substance which was then found to show an action similar to that of the azo-dye prontosil in protecting mice against streptococci. This finding was confirmed by Goissidet, Despois, Gailliot and Mayer (1936), and by Buttle, Gray and Stephenson (1936). Finally Kellner (1936) brought forward some evidence, and later Fuller (1937) conclusively proved, that *p*-aminobenzenesulphonamide is formed in the tissues when prontosil is given to animals and man by mouth. *p*-Aminobenzenesulphonamide has of course been known for the past thirty years, having been prepared by Gelmo in 1908.

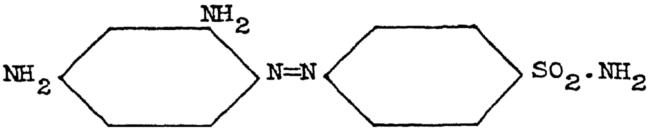
Since then sulphonamides and related compounds have been subjected to intensive study on the part of chemists, experimental pathologists and clinicians. Many new compounds have been produced and a flood of communications on the subject has already appeared.

The compounds which so far have been used with any frequency in therapeutics are as follows:—

(1) **Prontosil** (originally termed streptozon and later prontosil rubrum, prontosil or sulphamido-chrysoidin). The fact that azo-dyes, that is compounds containing the linkage —N : N—, are

possessed of bactericidal properties was first demonstrated by Eisenberg (1913), who found that chrysoidin, *m*-diaminoazobenzene, killed streptococci *in vitro.* Unfortunately no therapeutic action could be obtained *in vivo.*

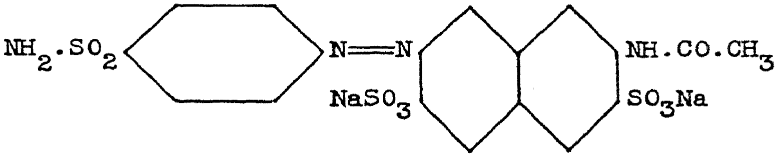
In the investigation of a number of azo compounds for anti-streptococcal activity a red azo dye was prepared by Mietzsch and Klarer (1932) by diazotising sulphanilamide and coupling *m*-phenylenediamine. This compound, 4-sulphonamido-2′ : 4′-diamino-azobenzene, was originally employed as the hydrochloride which is only soluble in water to the extent of 1 in 400. Later the base, which is still less soluble in water, was used. The absorption of the two compounds is practically identical, but the base does not produce such intense staining. The base is easily soluble in acetone (14·1 per cent.), moderately soluble in alcohol (0·55 per cent. in 90 per cent. and 0·38 per cent. in absolute alcohol), and soluble to about 9 per cent. in a mixture of 95 per cent. alcohol and acetone in equal parts at 20° C. It also dissolves readily in pyridine and methanol. The addition of dilute hydrochloric acid to the base forms the hydrochloride, which has a melting point of 248°–250° C. and dissolves with difficulty in cold but more easily in hot water.



Prontosil

Prontosil is given almost entirely by mouth in tablets containing 0·5 gm. ; on rare occasions it has been used in a saturated solution in equal quantities of alcohol and acetone for application to the skin.

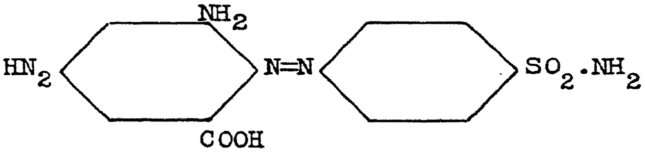
(2) **Prontosil Soluble** (soluble prontosil, neoprontosil, streptozon S, marketed by one firm in U.S.A. as prontosil or prontosil II) is the disodium salt of 4′-sulphonamidophenylazo-1-hydroxy-7-acetylaminonaphthalene-3 : 6-disulphonic acid. This compound, which is



Prontosil soluble

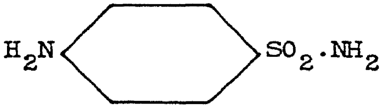
given intramuscularly, was first introduced as a 2·5 per cent. solution, but more recently a 5 per cent. solution has been prepared. In a dry state it is said to be soluble in absolute alcohol, ether, acetone, chloroform and benzol.

(3) **Rubiazol** * was prepared in France by Girard, and was first described by Levaditi and Vaisman (1935 [2]), and Gley and Girard (1936): it is stated to be 6-carboxy-4-sulphamido-2′-4′-diamino-azobenzene and thus differs from prontosil in possessing a COOH group. It is usually given orally in tablets containing 0·2 gm. or intramuscularly in doses of 5 c.c. of a 5 per cent. solution.



Rubiazol

(4) **Sulphanilamide,** *p*-aminobenzene sulphonamide, possesses the following synonyms, though this list makes no pretensions to



Sulphanilamide

---

* Some confusion has been caused by the fact that in France prontosil itself was at first called rubiazol: the change of nomenclature was made at the end of 1936 (cf. Poulain, 1936).

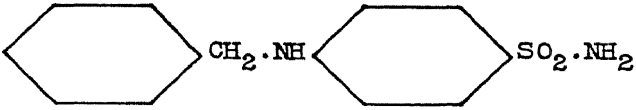
completeness : sulphonamide-P, colsulanyde, streptocide (Great Britain), sulfanilamide N.N.R., prontylin, sulfamidyl (U.S.A.), 1162F, septoplix, neococcyl, lysococcine (France), prontosil album (Germany), ergaseptine (Holland), astreptine (Belgium), supron (Poland), deseptyl, proseptol, ambeside (Hungary), proseptine (Roumania), streptazol (Albania), septamide (Palestine), stopton album (Argentina), streptoclase (Brazil) and therapol (Japan).

Sulphanilamide is a white crystalline powder with a bitter taste and no perceptible odour, soluble in acetone and dilute hydrochloric acid. In cold water its solubility is 0·5–0·8 per cent., but in boiling water its solubility is much increased : at 37° C. 1·5 gm. dissolves in 100 c.c. of water. An aqueous solution is neutral to litmus. It melts between 165° C. and 166·5 C., but in the standards laid down in America for inclusion in New and Non-official Remedies the melting-points are given as from 165° C. to 167° C. It should not contain ionisable halogen or sulphate, free ammonia, arsenic or heavy metals. It should contain not less than 99 and not more than 100·5 per cent. of $C_6H_8O_2N_2S$ when titrated in ice-cold hydrochloric acid solution with N/10 sodium nitrite until an immediate blue colour is given when spotted on starch-iodide paper ; 1 c.c. of N/10 sodium nitrite being taken as equal to 0·0172 gm. of $C_6H_8O_2N_2S$. Sulphanilamide can be quantitatively determined from the fact that it forms a well-defined dibromo-substitution product ; *p*-aminobenzenesulphonamide is treated with excess of potassium bromate in the presence of potassium bromide and hydrochloric acid and the excess of bromide is determined iodometrically. Sulphanilamide may also be determined by hydrolysing with 70 per cent. sulphuric acid and subsequently estimating the ammonia formed. The sulphur content should be not less than 18·3 per cent. and not more than 18·9 per cent. as determined by oxidising with potassium permanganate solution, acidifying with hydrochloric acid, precipitating and weighing as barium sulphate.

According to Fitch (1937) the best method of preparing a solution for parenteral administration is to dissolve at 80° C. 1 gm. of the drug in 80 c.c. of distilled water containing 0·5 gm. of sodium chloride. After sterilisation in the autoclave at 115°–116° C. for thirty minutes the solution is allowed to cool to body temperature before injection. If the temperature falls below about 37° C. the sulphanilamide will begin to crystallise out.

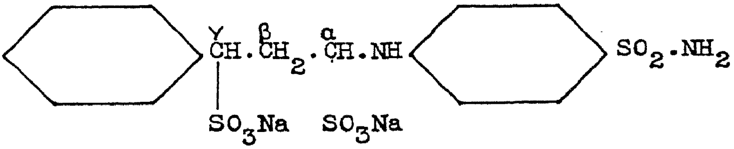
Other compounds for which more limited therapeutic data are available are :—

(*a*) Proseptasine (46 R.P., septazine, setazine). Proseptasine is dispensed in tablets of 0·5 gm. for oral administration.



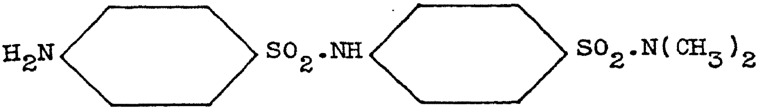
p-*Benzylaminobenzenesulphonamide*

(*b*) Soluseptasine (40 R.P.). Soluseptasine is put up for injection in the form of a 10 per cent. solution.

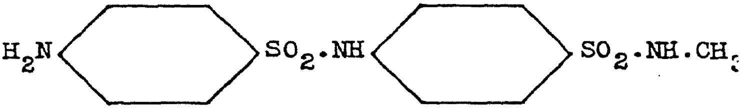


Disodium-p-(*γ-phenylpropylamino*)-*benzene sulphonamide-αγ-disulphonate*

(*c*) Uleron (DB 90, diseptal A, uliron), *p*-aminobenzenesulphonyl-*p′*-aminobenzenesulphondimethylamide, is a white substance, dispensed in tablets of 0·5 gm. for oral administration. It is practically insoluble in water but soluble in alkalis and acetone.

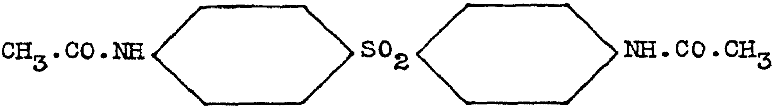


p-*Aminobenzenesulphonyl*-p′-*aminobenzenesulphondimethylamide* (*d*) Diseptal B (DB 87) is also made up in tablets of 0·5 gm. for oral administration in the treatment of gonorrhœa.



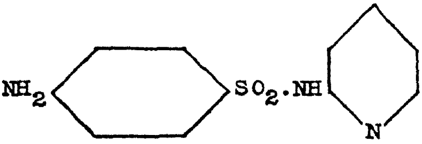
p-*Aminobenzenesulphonyl*-p′-*aminobenzenesulphonmethylamide*

(*e*) Rodilone (1399F). This compound has been used to a small extent in France in the treatment of gonorrhœa (*cf.* p. 403).



4 : 4′-*Diacetylaminodiphenylsulphone*

(*f*) 2-(*p*-Aminobenzenesulphonamido)-pyridine (M. & B. 693), prepared by Ewins, was found by Whitby (1938) to be active in pneumococcal infections in mice. It is now being tested therapeutically in pneumococcal infections in man (*cf.* p. 386).



2-(p-*Aminobenzenesulphonamido*)-*pyridine*

References

Buttle, G. A. H., Gray, W. H., and Stephenson, D., 1936, Protection of mice against streptococcal and other infections by *p*-aminobenzenesulphonamide and related substances. *Lancet*, *i.*, 1286.

Domagk, G., 1935 [1], Ein Beitrag zur Chemotherapie der bakteriellen Infektionen. *Dtsch. med. Wschr.*, LXI., 250.

Domagk, G., 1935 [2], Eine neue Klasse von Desinfektionsmitteln. *Ibid.*, LXI. 928.

Eisenberg, P., 1913, Untersuchungen über halbspezifische Desinfektionsvorgänge. I. Ueber die Wirkung von Farbstoffen auf Bakterien. *Zbl. Bakt.*, Abt. I, Orig., LXXI., 420.

Fitch, W. K., 1937, The nomenclature and dosage of new antistreptococcal preparations. *Practitioner*, CXXXIX., 680.

Foerster, 1933, Sepsis in Anschluss an ausgedehnte Periporitis. Heilung durch Streptozon. *Zbl. Haut- u. GeschlKr.*, XLV., 549.

Fuller, A. T., 1937, Is *p*-aminobenzenesulfonamide the active agent in prontosil therapy ? *Lancet, i.*, 194.

Gelmo, P., 1908, Über Sulfamide der *p*-Amidobenzolsulfonsäure. *J. prakt. Chem.*, LXXVII., 369.

Gley, P., and Girard, A., 1936, Un nouveau dérivé de la sulfamidochrysoidine très actif contre l'infection streptococcique. *Pr. méd.* XLIV., 1775.

Goissidet, P., Despois, R., Gailliot, P., and Mayer, R., 1936, De l'action du radical sulfamide : SO_2NH_2 sur l'infection streptococcique expérimentale de la souris. *C. R. Soc. Biol., Paris*, CXXI., 1082.

Grütz, O., 1934, Beitrag zur Kenntnis der infektiöstoxischen, Erythemaexsudativum-multiforme-ähnlichen Exantheme. *Med. Klinik*, XXX., 52.

Heidelberger, M., and Jacobs, W. A., 1919, Synthesis in the cinchona series. III. Azo dyes derived from hydrocupreine and hydrocupreidine *J. Amer. Chem. Soc.*, XLI., 2131.

Hörlein, H., 1937, The development of chemotherapy for bacterial infections. *Practitioner*, CXXXIX., 635.

Hörlein, H., Dressel, O., and Kothe, H., 1909, Deutsche Reichspatente 226,239 : 226, 240 and 230,594.

Hörlein, H., Dressel, O., and Kothe, H., 1910, Deutsches Reichspatent 235,775.

Kellner, P., 1936, Etude sur l'élimination de la sulfamido-chrysoidine. Thèse, Paris.

Levaditi, C., and Vaisman, A., 1935[1], Action curative et préventive du chlorhydrate de 4′-sulfamido-2,4-diaminoazobenzène dans l'infection streptococcique expérimentale. *C. R. Acad. Sci., Paris*, CC., 1694.

Levaditi, C., and Vaisman, A., 1935[2], Action curative et préventive du chlorhydrate de 4′-sulfamido-2,4-diaminoazobenzène et de quelques dérivés similaires dans la streptococcie expérimentale. *Pr. méd.*, XLIII., 2097.

Mietzsch, F., and Klarer, I., 1932, Deutsches Reichspatent 607,537.

Morgenroth, J., and Levy, R., 1911, Chemotherapie der Pneumokokkeninfektion. *Berl. klin. Wschr.*, XLVIII., 1560 and 1979.

Nitti, F., and Bovet, D., 1935, Action du 4-sulfonamide-2,4-diaminobenzol (prontosil) sur des infections streptococciques de la souris provoquées par des streptocoques d'origine humaine. *C. R. Soc. Biol., Paris*, CXIX. 1277.

Poulain, P., 1936, Contribution à l'étude expérimentale de la sulfamidochrysoidine et de quelques-uns de ses dérivés. Thèse, Paris.

Tréfouël, J., Tréfouël, Mme., Nitti, F., and Bovet, D., 1935, Activité du *p*-amino-phényl-sulfamide sur les infections streptococciques expérimentales de la souris et du lapin. *C. R. Soc. Biol., Paris*, CXX., 756.

Whitby, L. H., 1938, Chemotherapy of pneumococcal and other infections with 2-(*p*-aminobenzenesulphonamido)-pyridine. *Lancet, i.*, 1210.

PHARMACOLOGY

Up to the present, extensive pharmacological observations have been carried out only on sulphanilamide, and to a lesser degree on prontosil, prontonsil soluble and uleron.

The investigations of Marshall, Emerson and Cutting (1937 [1 & 2]) have shown that when given by mouth in doses of 0·05 to 0·2 gm. per kilogram of body weight sulphanilamide is rapidly absorbed from the gastro-intestinal tract, the process being complete in three to four hours. Subcutaneous injection does not lead to a higher concentration in the blood than does oral administration, while after intravenous injection of 0·1 gm. per kilogram of body weight the rate of elimination is the same as after oral administration. After oral administration of a single dose a peak in the blood concentration is reached in about three hours, after which the amount decreases uniformly until at the end of twenty-four hours no further evidence of its presence is obtainable. This prolonged persistence in the blood is, as Garrod (1938) points out, an important factor in its successful action, since other substances theoretically capable of chemotherapeutic action are so rapidly excreted that an adequate blood level is not maintained. By repeated administration at intervals of from four to six hours concentrations of 1 in 5,000 to 1 in 10,000 can be easily maintained in the blood. The concentration of sulphanilamide in the blood, both in the free and acetyl form, resulting from a given per pound dosage, is found to show very considerable variation in different individuals (Stewart, Rourke and Allen, 1938). There is some evidence that in animals such as the cow and the horse administration by mouth leads to a higher blood concentration than when the drug is injected subcutaneously in suspension. Persistence in the blood is apparently due to the re-absorption in the renal tubules of from 70 to 80 per cent. of the sulphanilamide in the glomerular filtrate. While the rate of clearance is independent of the plasma level, it increases with the rate of flow of the urine. Thus impaired renal function may decrease the rate of excretion, while increased diuresis will help to remove the drug from the circulation in cases where toxic effects have occurred. As a rule, after administration of a given daily dose of sulphanilamide in divided doses, it takes from two to three days to establish equilibrium between the amount ingested and the amount excreted and about the same time to free the tissues from the drug. In normal mice 65 per cent. of injected sulphanilamide is found in the urine and in infected mice 46 per cent. (Fuller, 1937). No explanation is available, but it is of interest that Long and Bliss (1937) showed that prontosil can be reduced by streptococci.

Similar results are obtained in the case of prontosil soluble, 51 per cent. of which is found in the urine after intramuscular or intravenous injection in normal animals, but only 38 per cent. in infected animals (Fuller, 1937). Prontosil itself is excreted more slowly. After a single dose of prontosil, sulphanilamide does not appear in the urine for from four to six hours. Fuller (1937) reported that of a dose of prontosil given in a 10 per cent. gum suspension, by mouth, to a mouse only a small fraction was excreted in five days. Colebrook and Kenny (1936) similarly found that a subcutaneous injection of 10 per cent. prontosil in aqueous suspension protected mice against an infection of streprococci four days later, while the mice continued to excrete prontosil for twenty-six days after receiving the drug. That the protective action is almost certainly due to the formation of sulphanilamide is shown by the fact that, if the molecular equivalent of 6 mgm. of sulphanilamide in terms of prontosil soluble (20·5 gm.) is injected parenterally into mice, the blood levels of free sulphanilamide at the end of four hours are essentially those obtained at the same period after the parenteral injection of 6 mgm. of sulphanilamide (Feinstone *et al.*, 1938).

Sulphanilamide is widely and uniformly distributed in the body except in bone and fat, which contain smaller amounts than other tissues. The concentration in plasma is slightly less than in whole blood. The drug is found in the normal body fluids such as the saliva, sweat, tears, bile and pancreatic fluids, as well as in pathological exudates in the pleural and peritoneal cavities. As might be expected, sulphanilamide is found in menstrual blood, but in the normal secretion of the cervix uteri it occurs in such small amounts that its bacteriostatic or bactericidal action is problematical. In rabbits, 4 to 8 per cent. of sulphanilamide is found within two hours in the bile. When given by mouth to pregnant rabbits, sulphanilamide passes into the amniotic fluid and from the maternal to the fœtal circulation. According to Adair *et al.* (1938), sulphanilamide produces an increase in fœtal mortality in rabbits. In man also sulphanilamide after oral administration is found in maternal and fœtal blood (Barker, 1938). The acetylated form is present in relatively lower concentration in the fœtal as compared with the maternal blood (Lee, Anderson and Chen, 1938). In cows, sulphanilamide given by mouth passes into the milk, the concentration being less than in blood and reaching a maximum somewhat later. In human milk twenty-four hours after oral administration from 0·55 to 2·17 mgm. per 100 c.c. were found by Adair *et al.* (1938). In milk sulphanilamide is excreted partly unchanged and partly in the acetyl form, while excretion continues for some time after the blood level has become negligible. According to Rambo (1938) sulphanilamide, orally administered to rabbits, is found in the eye in the aqueous, vitreous and lens. Of special interest and importance is the passage through the blood-brain barrier into the cerebrospinal fluid. Sulphanilamide was found by Marshall, Emerson and Cutting (1937 [1]) to pass readily into the cerebrospinal fluid of healthy dogs and human beings, while the same observers noted no difference in the passage of sulphanilamide into the cerebrospinal fluid of normal human beings and of those suffering from meningococcal meningitis. The curve of concentration in the cerebrospinal fluid is parallel to that in the blood at about three-quarters of the blood level (*cf.* Proom and Buttle, 1937). Marshall and Cutting (1938), however, showed that in healthy dogs prontosil soluble only passed very slowly from the blood to the cerebrospinal fluid. This confirms the previous observations of Jauerneck and Gueffroy (1937) and Millett (1937), who found that the red dye-stuffs, prontosil and prontosil soluble, passed only slowly from the blood to the cerebrospinal fluid of patients with meningococcal meningitis, while sulphanilamide passed over much more rapidly. The cerebrospinal fluid of rabbits given large doses of sulphanilamide was shown by Proom (1937) to contain sufficient of the drug to inhibit the growth of a small inoculum of streptococci, whereas the same number of organisms grew freely in the normal fluid. Mellon and Bambas (1937) made similar observations in man. Further observations are required on the relative rate of passage of these compounds into the cerebrospinal fluid in health and in meningococcal meningitis.

Allott (1938) has found that in patients with meningococcal meningitis there is considerable variation in the rapidity with which an effective concentration of sulphanilamide is reached in the cerebrospinal fluid and in the level maintained, the dose of the drug administered being kept constant.

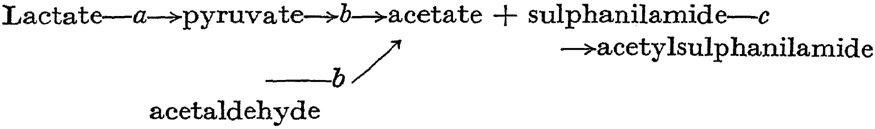
Apart from small amounts excreted in the saliva and sweat, sulphanilamide is wholly excreted in the urine. In man there is some evidence to show that in old age the rate of excretion of sulphanilamide is reduced. In dogs sulphanilamide is excreted in the urine unchanged, while in rabbits it is in part converted into *p*-acetylaminobenzenesulphonamide, which is therapeutically inactive. The same is true of man, monkey and mouse (*cf.* Fuller, 1937, and Marshall, Cutting and Emerson, 1937 ¹). In the mouse the rate of excretion of sulphanilamide is much more rapid than in the rabbit, monkey or man. In man after single doses of sulphanilamide multiple peaks occur in the concentration and rate of output of free sulphanilamide and its acetyl derivative in the urine. The rate and concentration maxima do not appear to be related (*cf.* Helmholz and Osterberg, 1937, and Scudi and Ratish, 1938). The acetyl derivative is slightly more toxic for animals than sulphanilamide itself (*cf.* p. 338), but only relatively small amounts of this derivative are found in the blood of patients treated with the drug (Marshall, 1938, and Marshall, Cutting and Emerson, 1938).

ABSORPTION OF SULPHANILAMIDE AND ACETYLSULPHANILAMIDE IN MICE (Marshall, Cutting and Emerson, 1938)

Time in hours after drug.	Mgm. per 100 c.c. in blood after			
	Sulphanilamide		Acetylsulphanilamide	
	Free	Total	Free	Total
4	110	113	3	35
8	126	134	2	26
24	38	57	5	27
30	—	—	0·3	3
48	—	—	0	0·6

Acetylsulphanilamide is much less soluble in water than sulphanilamide itself, while if given to the dog, rabbit or mouse a portion is changed back to sulphanilamide (Marshall, Cutting and Emerson, 1938). According to Harris and Klein (1938), acetylation of sulphanilamide is brought about *in vitro* by liver tissue but not by spleen, kidney or blood. The intact liver cell is essential. While liver tissue from rabbits was usually very active in bringing about conjugation, only two of four rat livers acted in this way. Conjugation varies greatly, however, even in animals of the same species. Acetylation is dependent on aerobic respiration, but the presence of sulphanilamide in concentrations up to 200 mgm. per cent. had no effect upon either aerobic or anaerobic tissue respiration.

Klein and Harris (1938) suggest the following scheme for the acetylation of sulphanilamide by rabbit liver :—



in which (*a*) is inhibited by anaerobiosis, thus limiting anaerobic acetylation, and (*b*) is either oxidation or dismutation. Conjugation (*c*) of acetate and sulphanilamide is not reversible, is inhibited by high concentrations of iodoacetamide and arsenious oxide and is only slightly inhibited by anaerobiosis.

Hawking (1937), using sulphanilamide, observed no pharmacological action on rabbit intestine, guinea-pig uterus or frog heart : in cats and dogs given 0·7 gm. per kilogram of body weight intravenously no rise in blood pressure was noted. With prontosil, Barke (1937) found that a contraction was produced in the cat's small intestine suspended in Ringer's solution : the addition of hydrochloric acid increased the contraction. A temporary decrease in renal function is observed after giving large doses of sulphanilamide, but no permanent kidney injury appears to result.

In studying the effects of sulfanilamide, Hartmann, Perley and Barnett (1938) have recently found that, though the alkali reserve falls and bicarbonate is excreted in the urine, the *p*H of the serum nevertheless remains constant or even rises. They suggest that the fall in the alkali reserve is a compensation for a gaseous alkalosis due to hyperpnœa, but exactly how the hyperpnœa is caused is uncertain, though it is possibly brought about through the Herring-Brown reflex, which is affected centrally. It is obvious that any attempt to interpret the variation in alkali reserve found in patients receiving sulphanilamide is beset with difficulty, for on the one hand true acidosis may develop if hyperpnœa ceases, while alkalosis may follow if alkali is given, as, for instance, in the treatment of urinary infections due to *Bacterium coli.*

References

ADAIR, F. L., HESSELTINE, H. C., and HAC., L. R., 1938, An experimental study of the behavior of sulfanilamide. *J. Amer. med. Ass.*, CXI., 766.

ALLOTT, E. N., 1938, Sulphanilamide content of cerebrospinal fluid during the treatment of meningococcal meningitis. *Lancet, ii.*, 13.

BARKE, A., 1937, Ueber die Wirkung von Prontosil auf den überlebenden Katzendarm. *Dtsch. tierärztl. Wschr.*, XLV., 509.

BARKER, R. H., 1938, The placental transfer of sulfanilamide. *New Eng. J. Med.*, CCXIX., 41

COLEBROOK, L., and KENNY, M., 1936, Treatment of human puerperal infections in mice with prontosil. *Lancet, i.*, 1279.

FEINSTONE, W. H., BLISS, E. A., OTT, E., and LONG, P. H., 1938, Observations concerning the toxicity, absorption and therapeutic effect of sulphanilamide and certain related organic sulphur-containing compounds in experimental infections in mice. *Bull. Johns Hopk. Hosp.*, LXII., 565.

FULLER, A. T., 1937, Is *p*-aminobenzenesulfonamide the active agent in prontosil therapy ? *Lancet, i.*, 194.

GARROD, L. P., 1938, The chemotherapy of bacterial infections. *Ibid., i.*, 1125.

HARRIS, J. S., and KLEIN, J. R., 1938, Acetylation of sulfanilamide by liver tissue *in vitro*. *Proc. Soc. exp. Biol. N.Y.*, XXXVIII., 78.

HARTMANN, A. F., PERLEY, A. M., and BARNETT, H. J., 1938, A study of some of the physiological effects of sulfanilamide. I. Changes in the acid base balance. *J. clin. Invest.*, XVIII., 465.

HAWKING, F., 1937, Pharmacological actions of sulphanilamide. *Lancet, ii.*, 1019.

HELMHOLZ, H. F., and OSTERBERG, A. E., 1937, The rate of excretion and bactericidal power of sulfanilamide in urine. *Proc. Mayo Clin.*, XII., 377.

JAUERNECK, A., and GUEFFROY, W., 1937, Prontosilnachweis im Liquor. *Klin. Wschr.*, XVI., 1544.

KLEIN, J. R., and HARRIS, J. S., 1938, The acetylation of sulfanilamide *in vitro*. *J. Biol. Chem.*, CXXIV., 613.

LEE, H. M., ANDERSON, R. C., and CHEN, K. K., 1938, Passage of sulphanilamide from mother to fetus. *Proc. Soc. exp. Biol., N.Y.*, XXXVIII., 366.

Long, P. H., and Bliss, E. A., 1937, Use of para-aminobenzene-sulphonamide (sulphanilamide) or its derivatives in treatment of infections due to beta-hemolytic streptococci, pneumococci and meningococci. *Sth. med. J., Nashville,* XXX., 479.

Marshall, E. K., Jr., 1938, Certain phases of the pharmacolytic properties of sulfanilamide. *Med. Ann. District of Colombia,* VII., 5.

Marshall, E. K., Jr., and Cutting, W. C., 1938, Sulphanilamide in bacterial meningitis. *Lancet, i.,* 1186.

Marshall, E. K., Jr., Cutting, W. C., and Emerson, K., Jr., 1937 [1], Acetylation of para-aminobenzenesulfonamide in the animal organism. *Science,* LXXXV., 202.

Marshall, E. K., Jr., Cutting, W. C., and Emerson, K., Jr., 1937 [2], The renal excretion of sulfanilamide. *J. Pharmacol.,* LXI., 191.

Marshall, E. K., Jr., Cutting, W. C., and Emerson, K., Jr., 1938, The toxicity of sulfanilamide. *J. Amer. med. Ass.,* CX., 252.

Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C., 1937 [1], Para-aminobenzenesulfonamide. Absorption and excretion: method of determination in urine and blood. *Ibid.,* CVIII., 953.

Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C., 1937 [2], The distribution of sulfanilamide in the organism. *J. Pharmacol.,* LXI., 196.

Mellon, R. R., and Bambas, L. L., 1937, Bacteriostatic effect of sulfanilamide in spinal fluid of convalescent cases of streptococcal meningitis. *Proc. Soc. exp. Biol., N.Y.,* XXXVI., 682.

Millett, J., 1937, Hemolytic streptococcus meningitis: report of a case treated with sulfanilamide and prontosil soluble intramuscularly and intrathecally with recovery. *New Engl. J. Med.,* CCXVII., 556.

Proom, H., 1937, The therapeutic action of *p*-aminobenzenesulphonamide in meningococcal infections of mice. *Lancet, i.,* 16.

Proom, H., and Buttle, G. A. H., 1937, *p*-Aminobenzenesulphonamide in bacterial meningitis. *Ibid., i.,* 661.

Rambo, V. C., 1938, The effects of sulfanilamide as determined in the eyes of rabbits. *Amer. J. Ophthal.,* XXI., 739.

Scudi, J. V., and Ratish, H. D., 1938, The urinary excretion of single doses of sulfanilamide. *J. Lab. clin. Med.,* XXIII., 615.

Stewart, J. D., Rourke, G. M., and Allen, J. G., 1938, Excretion of sulfanilamide. *J. Amer. med. Ass.,* CX., 1885.

TOXICITY FOR ANIMALS

A number of observations have been made on the toxicity of sulphanilamide administered to animals by different routes. Buttle, Gray and Stephenson (1936), giving the drug in gum acacia suspension by mouth, state that 2·5 gm. per kilogram is innocuous, 4 gm. is tolerated but causes inco-ordination and paralysis, 5 gm. kills two of six mice and 10 gm. kills six of six mice. Rosenthal (1937) reports that given subcutaneously in olive oil suspension, 4 gm. per kilogram killed none of five mice, 6 gm. per kilogram killed three of four mice. Chen (1938), by intravenous injection, found that the dose lethal for 50 per cent. of mice, that is to say the $L.D._{50}$ (*cf.* Trevan, 1927), was from 0·35 to 0·40 gm. per kilogram of body weight. Halpern and Mayer (1937) found that by oral administration the $L.D._{50}$ was 6 gm. per kilogram for mice. Barlow (1937) obtained similar results by oral administration. Subcutaneously in an isotonic aqueous menstruum the M.L.D. lay between 6 and 8 gm. per kilogram of body weight. Sulphanilamide in water, 20 per cent. gum acacia and olive oil was toxic in doses per kilogram of 2·75, 3·75 and 4 gm. respectively. Marshall, Cutting and Emerson (1938) report a somewhat lower degree of toxicity, in that orally for mice the $L.D._{50}$ was 3·8 gm. per kilogram and the $L.D._{100}$ over 10 gm. per kilogram, while 0·9 gm. per kilogram would kill one mouse in 10,000. Feinstone *et al.* (1938) agree closely with Marshall *et al.* (1938), since they found that orally in mice 3 gm. per kilogram is innocuous, 3·5 gm. kills 80 per cent. and 4 gm. 92 per cent.

Dyke (1937) compared the relative oral toxicities for mice of pure *o*-, *m*- and *p*-aminobenzenesulphonamides. The $L.D._{50}$ per kilogram of body weight were 3·0–4·0 gm., 4·5–5·0 gm. and 4·0 gm. respectively. From this it may be inferred that the presence of traces of *o*- and *m*-aminobenzenesulphonamides in commercial specimens of *p*-aminobenzenesulphonamide does not appreciably affect the toxicity of the latter.

The toxicity of acetylsulphanilamide appears to be somewhat greater, as seen in the table.

TOXICITY OF SULPHANILAMIDE AND ACETYLSULPHANILAMIDE FOR MICE BY MOUTH (Marshall, Cutting and Emerson, 1938)

Dose in gm. per kilogram	Sulphanilamide		Acetylsulphanilamide	
	No. of mice	Per cent. died	No. of mice	Per cent. died
1	12	8·3	12	0·0
2	28	7·1	20	45·0
3	24	25·0	23	57·0
4	20	65·0	20	70·0
5	—	—	12	75·0
6	24	88·0	24	92·0

For rats, Halpern and Mayer (1937) report the L.D.$_{90}$ of sulphanilamide as 4 gm. per kilogram, given by mouth. Domagk (1935) found 3 gm. per kilogram tolerated without ill effect. For rabbits the toxic dose *per os* was 2 gm. per kilogram. Raiziss and his co-workers (1937) reported the L.D.$_{50}$ for rabbits on oral administration as 2 gm. and the L.D.$_{100}$ as 2·5 gm. per kilogram. Marshall, Cutting and Emerson (1938), giving sulphanilamide in gum acacia by mouth, found that five of seven rabbits given 1 gm. per kilogram showed symptoms, while two rabbits receiving 3·5 gm. per kilogram died in the course of two days.

For dogs, Halpern and Mayer (1937) found the toxic dose of sulphanilamide given by mouth to be 1 gm. per kilogram. Marshall, Cutting and Emerson (1938) found that 0·1 and 0·2 gm. caused no symptoms in dogs, 0·5 gm. in one of two dogs, and 1 gm. per kilogram by mouth caused symptoms in six of seven dogs, but the animals recovered. Of nine dogs given 1·6 to 2 gm. per kilogram, all showed symptoms and one animal died. Two dogs given 5 gm. per kilogram for four days and a third 6 gm. per kilogram for five days recovered completely. In cats, 1 gm. per kilogram was found by Domagk (1935) to be the minimum lethal dose, while for guinea-pigs it was 2·5 gm. per kilogram of body weight. In a limited number of experiments dogs and rats showed no signs of chronic toxicity after therapeutic doses and no pathological lesions after prolonged administration of sulphanilamide.

The acetyl derivative in doses of 0·1 to 0·2 gm. per kilogram caused no symptoms in dogs, but of seven dogs receiving 2 gm. per kilogram two showed symptoms and one died on the fourth day; 2 gm. *per os* caused no symptoms in rabbits.

The toxicity of prontosil was investigated by Domagk (1935), who stated that rabbits and mice tolerated 0·5 gm. per kilogram by mouth and subcutaneously. Rats tolerated 3 gm. per kilogram without upset. For mice it is doubtful whether the oral lethal dosage can be calculated, owing to high tolerance and the limited gastric capacity of the species. In cats 1 gm. per kilogram was vomited without further disturbance. In guinea-pigs the borderline toxic dose was 1 gm. per kilogram of body weight. Prontosil soluble is found to be harmless to mice and guinea-pigs in oral doses of 3 gm. per kilogram. Feinstone *et al.* (1938) gave mice up to 12 gm. per kilogram of body weight without causing death. On intravenous injection mice tolerate not more than 1·25 gm. per kilogram, while in cats and guinea-pigs the intramuscular injection of 3 gm. per kilogram causes vomiting in cats and some irregularity of temperature in both species. Dogs tolerate 2 gm. per kilogram injected intramuscularly.

Data on the toxicity of proseptasine and soluseptasine are not extensive. Proseptasine is said to be completely non-toxic for mice, while soluseptasine is slightly toxic to mice in doses of 5 gm. per kilogram (Whitby, 1937; *cf.* also Feinstone *et al.*, 1938).

Uleron, it is claimed, has a very low toxicity for mice on oral administration, and as much as 800 mgm. has been given to a 20 gm. mouse without causing symptoms. Its acute toxicity by the oral route cannot, however, be determined because of its low rate of absorption.

The toxic symptoms produced by sulphanilamide in animals are referable mainly to the central nervous system. In mice inco-ordination and paralysis are seen, while in rabbits Hawking (1937) found that doses of 1·5 to 2 gm. per kilogram of body weight caused weakness of the legs, dyspnœa and a rigid extension of the limbs, with contraction of the head resembling decerebrate rigidity. In three animals degenerative changes were found in the anterior horn cells of the spinal cord and in some of the cortical neurones, but in animals that recovered no pathological lesions were found in the liver, kidneys and viscera.

In dogs sulphanilamide in doses of 1 gm. per kilogram causes salivation, vomiting, diarrhœa, hyperpnœa, excitement, muscular weakness, ataxia and finally spastic rigidity of the limbs and decreasing sensation. The dogs appear to be blind, and the placing reactions known to be due to the cerebral cortex are absent (Marshall, Cutting and Emerson, 1938).

Studies of the pathological lesions produced by sulphanilamide have been few. Hageman (1937), however, observed, after intraperitoneal inoculation of sulphanilamide in mice, a fibroblastic foreign body reaction to crystals of the drug on the surface of the liver, spleen and kidney. Hæmosiderin was deposited in the spleen in the areas adjacent to the Malpighian bodies: in the bone marrow there was an increased production of eosinophils. Rimington and Hemmings (1938) found that in rats treated with sulphanilamide the spleens were blackish-brown in colour and much engorged. Deposits of a greenish-brown granular pigment were seen histologically, together with considerable quantities of stainable iron. Oakley (1937) noted that when single doses, such as 40 mgm. or repeated small doses, of prontosil are given by mouth to mice the majority of the animals become deeply pigmented and not uncommonly die. At death, apart from the generalised orange-red pigmentation and the presence of the drug in the bladder and gall-bladder, the only striking change is in the kidneys, which are much enlarged. Microscopically the dye is seen as concretions of varying size in the straight and convoluted tubules, which are greatly dilated from obstruction, often as far back as Bowman's capsule. Stewart *et al.* (1938) point out that sulphanilamide is precipitated in urine at room temperature and suggest the possibility of calculus formation. When injected in the form of a concentrated aqueous solution into the anterior chamber of the eye, no pathological lesions result, although the power of accommodation is lost for some days: placed in the conjunctival sac there is no reaction (Rambo, 1938).

References

BARLOW, O. W., 1937, Relative toxicities and therapeutic values of three chemotherapeutic agents of the sulphonamide type. *Proc. Soc. exp. Biol., N.Y.*, XXXVII., 315.

BUTTLE, G. A. H., GRAY, W. H., and STEPHENSON, D., 1936, Protection of mice against streptococcal and other infections by *p*-aminobenzenesulphonamide and related substances. *Lancet, i.*, 1286.

CHEN, K. K., 1938, quoted by Marshall, E. K., Jr., Cutting, W. C., and Emerson, K. *J. Amer. med. Ass.*, CX., 252.

DOMAGK, G., 1935, Ein Beitrag zur Chemotherapie der bakteriellen Infektionen. *Dtsch. med. Wschr.*, LXI., 250.

DYKE, W. J. C., 1937, The relative oral toxicities for mice of the isomeric aminobenzenesulphonamides. *Quart. J. Pharm.*, X., 319.

FEINSTONE, W. H., BLISS, E. A., OTT, E., and LONG, P. H., 1938, Observations concerning the toxicity, absorption and therapeutic effect of sulphanilamide and certain related organic sulphur-containing compounds in experimental infections in mice. *Bull. Johns Hopk. Hosp.*, LXII., 565.

HAGEMAN, P. O., 1937, Toxicity of sulfanilamide. A study of pathological lesions in white mice. *Proc. Soc. exp. Biol., N.Y.*, XXXVII., 119.

HALPERN, B. N., and MAYER, R. L., 1937, Toxicité expérimentale comparée de quelques substances anti-streptococciques. *Pr. méd.*, XL., 747.

HAWKING, F., 1937, Pharmacological actions of sulphanilamide. *Lancet, ii.*, 1019.

MARSHALL, E. K., Jr., CUTTING, W. C., and EMERSON, K., Jr., 1938, The toxicity of sulfanilamide. *J. Amer. med. Ass.*, CX., 252.

OAKLEY, C. L., 1937, Note on " prontosil " poisoning in mice. *Biochem. J.*, XXXI., 729.

RAIZISS, G. W., SEVERAC, M., and MOETSCH, J. C., 1937, Chemotherapeutic studies of sulfamidyl (para-aminobenzenesulfonamide) in experimental beta-hemolytic streptococcic infections. *J. Chemother.*, XIV., 1.

RAMBO, V. C., 1938, The effects of sulphanilamide as determined in the eyes of rabbits. *Amer. J. Ophthal.*, XXI., 739.

RIMINGTON, C., and HEMMINGS, A. W., 1938, Porphyrinuria following sulphanilamide : sulphanilamide dermatitis. *Lancet, i.*, 770.

ROSENTHAL, S. M., 1937, Studies in chemotherapy : chemotherapy of experimental pneumococcus infections. *Publ. Hlth. Rep., Wash.*, LII., 48.

STEWART, J. D., ROURKE, G. M., and ALLEN, J. G., 1938, Excretion of sulfanilamide. *J. Amer. med. Ass.*, CX., 1885.

TREVAN, J. W., 1927, The error of determination of toxicity. *Proc. roy. Soc.*, B., CI., 483.

WHITBY, L. E. H., 1937, An experimental assessment of the therapeutic efficacy of amino compounds with special reference to *p*-benzylaminobenzenesulphonamide. *Lancet, i.*, 1517.

THE ESTIMATION OF SULPHANILAMIDE IN THE TISSUES AND BODY FLUIDS

Several methods have now been devised for the estimation of prontosil and sulphanilamide in the tissues and body fluids. The earliest tests were those elaborated by Fuller (1937) and by Marshall *et al.* (1937). Fuller (1937) found that both prontosil and prontosil soluble can be estimated in the urine by comparing the colour of the urine with that of a solution of the corresponding compound of known strength and the same *p*H. Prontosil soluble can be estimated in the presence of prontosil, when the colour of the latter is negligible compared with that of the first, but no method has been found of estimating the second when the first is present. Sulphanilamide is estimated in blood by adding trichloracetic acid, filtering, and to the filtrate adding one drop of 0·5 per cent. sodium nitrite. After a few minutes one drop of 1 per cent. alkaline thymol and 0·2 c.c. of 40 per cent. caustic soda are added: the colour is compared with suitable standards. The method described by Marshall, Emerson and Cutting (1937) and Marshall (1937 $^{1 \& 2}$), and applicable to blood, urine, cerebrospinal and other body fluids, consisted of diazotisation of the *p*-aminobenzenesulphonamide with nitrous acid and coupling the resulting diazo compound in acid solution with dimethyl-α-naphthylamine to produce a purplish red azo dye, which can be estimated colorimetrically. The reaction, which is said to be sufficiently delicate to distinguish one part in 20 millions in water, depends on the presence of an amino group substituted in the benzene ring, and will estimate any compound to which the sulphonamide compound is changed in the organism, provided the amino group is intact.

Certain modifications have been introduced by Marshall and Litchfield (1938). By destroying the excess nitrous acid after diazotisation and buffering the solution before coupling with dimethyl-α-naphthylamine, more rapid colour development and more permanent colours are obtained. The revised procedure for determining free sulphanilamide is as follows: to 10 c.c. of blood filtrate or acidified standard solution, 1 c.c. of a 0·1 per cent. freshly prepared solution of sodium nitrite is added. After three minutes' interval, 1 c.c. of a 1 M sodium dihydrogen phosphate solution containing 0·5 per cent. of ammonium sulphamate ($NaH_2PO_4 . H_2O$ 13·8 gm.: ammonium sulphamate 0·5 gm.: water 100 c.c.) is added, and, after two minutes' standing, 5 c.c. of the alcoholic solution of dimethyl-α-naphthylamine are added. After ten minutes the solutions are compared in a colorimeter. To determine the total sulphanilamide 10 c.c. of filtrate are treated with 1 c.c. of 2 N hydrochloric acid, heated in a boiling water-bath for one hour, cooled and the volume adjusted to 10 c.c. The subsequent procedure is as before except that a 2 M phosphate buffer containing 0·5 per cent. of ammonium sulphamate is used. For determining both free and conjugated sulphanilamide in blood a 1 : 20 is preferred to a 1 : 10 dilution.

In the method described by Proom (1938), which is a combination of those suggested by Fuller (1937) and Marshall *et al.* (1937), "1 c.c. of blood or body fluid is diluted with 2 c.c. of distilled water: 1 c.c. of a 20 per cent. aqueous solution of trichloracetic acid is then added and the mixture filtered through a small filter paper. To 1 c.c. of the filtrate is added 0·05 c.c. (1 c.c. of 0·5 per cent.) sodium nitrite solution. After three minutes 0·5 c.c. of a 1 per cent. alcoholic solution of dimethyl-α-naphthylamine is also added and the whole mixed by gentle shaking. After standing for fifteen minutes to allow the colour to reach its maximum intensity, the colour is compared with that obtained by treating in a similar manner a standard solution containing known quantities of sulphanilamide." The comparison may be done with any of the usual forms of colorimeter, while permanent colour standards can be prepared.

Schmidt (1938[1]), for estimating sulphanilamide in blood, has employed the tungstic acid blood filtrate obtained by the Folin-Wu technique, to which is added sodium-β-naphthoquinone-4-sulphonate. A similar method (Schmidt, 1938[2]) can be applied to the estimation of sulphanilamide in cerebrospinal fluid. The 0·05 per cent. solution of sodium-β-naphthoquinone-4-sulphonate must be freshly prepared: 1 c.c. is added to each of a series of standard tubes containing from 0·01 to 0·20 mgm. of sulphanilamide in 10 c.c. of distilled water made acid with one drop of 1·5 N hydrochloric acid. To 1 c.c. of the spinal fluid to be tested, diluted to 10 c.c. with distilled water and acidified, is added 1 c.c. of the naphthoquinonesulphonate solution. All tubes are placed in the dark for one hour and the results read in the colorimeter against the standard that matches most closely.

Instead of coupling with dimethyl-α-naphthylamine, Doble and Geiger (1938) employ diphenylamine : a difference in concentration of 0·1 mgm. sulphanilamide per 100 c.c. of solution can, it is claimed, be detected.

A micromethod, applicable to the blood and urine, and involving slight modifications in Marshall's method, is described by MacLachlan, Carey & Butler (1938).

Uleron can be estimated by the above methods, the standard solution of sulphanilamide being replaced, of course, by one of uleron.

References

DOBLE, J., and GEIGER, J. C., 1938, The determination of para-aminobenzenesulfonamide (sulfanilamide) in urine and blood. *J. Lab. clin. Med.*, XXIII., 651.

FULLER, A. T., 1937, Is *p*-aminobenzenesulphonamide the active agent in prontosil therapy ? *Lancet, i.*, 194.

MACLACHLAN, E. A., CAREY, B. W., and BUTLER, A. M., 1938, The determination of para-aminobenzenesulfonamide. *J. Lab. clin. Med.*, XXIII., 1273.

MARSHALL, E. K., Jr., 1937 [1], Determination of sulfanilamide in blood and urine. *Proc. Soc. exp. Biol., N.Y.*, XXVI., 422.

MARSHALL, E. K., Jr., 1937 [2], Determination of sulfanilamide in blood and urine. *J. Biol. Chem.*, CXXII., 263.

MARSHALL, E. K., Jr., EMERSON, K., and CUTTING, W. C., 1937, Para-aminobenzenesulfonamide. Absorption and excretion : method of determination in urine and blood. *J. Amer. med. Ass.*, CVIII., 953.

MARSHALL, E. K., Jr., and LITCHFIELD, J. T., Jr., 1938, The determination of sulfanilamide. *Science*, LXXXVIII., 85.

PROOM, H., 1938, Estimation of sulphanilamide in blood and other body fluids. *Lancet, i.*, 260.

SCHMIDT, E. G., 1938[1], The determination of sulfanilamide in tungstic acid blood filtrates by means of sodium-β-naphthoquinone-4-sulfonate. *J. Biol. Chem.*, CXXII., 757.

SCHMIDT, E. G., 1938 [2], The determination of sulfanilamide in cerebrospinal fluid by means of sodium-beta-naphthoquinone-4-sulfonate. *J. Lab. clin. Med.*, XXIII., 648.

TREATMENT OF INFECTIONS WITH STREPTOCOCCI BY PRONTOSIL AND SULPHANILAMIDE

(a) Experimental Investigations

Sufficient experimental evidence of the effects of prontosil and sulphanilamide on streptococcal infections in laboratory animals has now accumulated to show that the best results are obtained with hæmolytic streptococci belonging to Group A of Lancefield's classification. Even with hæmolytic streptococci of this type, however, differences are met with in the reaction of different strains, virulent being, as a rule, more susceptible than relatively avirulent strains. One strain of Group B (Aronson Neufeld) was highly susceptible to sulphanilamide (Buttle, Gray and Stephenson, 1936), but with others, infections of only a few lethal doses can be cured in mice. Hæmolytic streptococci of Group C are also less susceptible to sulphanilamide. Seastone (1937) found that one strain of a Group C organism which produced a chronic infection was but little affected once it had become established. Inoculated simultaneously with sulphanilamide the development of the streptococcus was either delayed or prevented. With another strain causing an acute disease sulphanilamide prevented infection altogether. Colebrook and Kenny (1936 [2]) cured one of two cases infected with a Group B streptococcus, two of Group C, and two of four infected with Group G type streptococci. Non-hæmolytic streptococci are much less susceptible than hæmolytic organisms, but even here there is some evidence that sulphanilamide may have a temporary restraining action on at least certain strains. Thus Muether and Kinsella (1938) produced an experimental endocarditis with non-hæmolytic streptococci in five dogs: after treatment with sulphanilamide only one died. Bliss and Long (1937) failed to cure urinary infections due to hæmolytic streptococci belonging to Lancefield's Group D of the *Str. viridans* type. A number of cases of subacute bacterial endocarditis due to *Str. viridans* in man have been treated with sulphanilamide. In some a temporary fall of temperature and a diminution in the number of organisms in the blood has occurred, but no permanent cure has been reported. *Streptococcus fœcalis* is highly resistant. Anaerobic hæmolytic streptococci have received little attention experimentally in regard to their reaction to prontosil or sulphanilamide, but in one patient, suffering from meningitis of otitic origin caused by anaerobic beta-hæmolytic streptococci, Smith *el al.* (1938) reported complete cure as a result of the administration of sulphanilamide by mouth. In three cases of puerperal fever, recorded by Colebrook and Purdie (1937), in which anaerobic streptococci were present in the blood, the results of treatment were entirely negative, but as the patients all had extensive thromboencephalitis they were hardly favourable subjects. Fortunately in man the majority of acute streptococcal infections are caused by organisms belonging to Group A.

Bosse (1936) believed that in splenectomised mice prontosil and sulphanilamide are less active in combating streptococcal infections. Gross, Cooper and Peebles (1937) were unable to confirm this observation. On the other hand, in the rat, deprivation of vitamins A and B does not interfere with the therapeutic action of sulphanilamide (Meunier and Nitti, 1938).

(b) Streptococcal Infections in Man

The effect of the sulphonamide group of drugs has now been tested on almost every form of streptococcal infection occurring in man. Extensive reviews have already been published by Long and Bliss (1937 [1]), Garrod (1938) and Bayer Products Ltd. (1938), so that only a brief résumé of the clinical evidence will here be attempted.

Puerperal Fever. By far the most extensive series of cases at present investigated is that reported from Queen Charlotte's Hospital, London. The first observations by Colebrook and Kenny (1936 [1, 2]) dealt with 64 cases treated with prontosil. The total daily dosage varied from 2 to 5·5 gm. at the beginning of treatment, the amount being reduced a day or two after the temperature fell. In 36 of the cases prontosil was considered to have played a part in causing recovery. Among the prontosil-treated cases only three deaths occurred. A series of 106 cases reported by Colebrook and Purdie (1937) were treated by sulphanilamide given either orally in doses up to 15 gm. a day, or by intravenous drip in cases where vomiting was severe. Of these cases all were due to hæmolytic streptococci except six, three of which were caused by non-hæmolytic streptococci and three by staphylococci. Among the sulphanilamide-treated cases eight deaths took place. The death rates for the two series were thus 4·7 per cent. and 8 per cent. respectively, a striking reduction on the previously reported death rate of 20 per cent. Even if this diminution in the death rate were due to a spontaneous diminution in the invasive character of the hæmolytic streptococci, this could hardly assist those cases in which infection spreads beyond the uterus. In an analysis of such cases Gibberd (1937) showed that the death rate was 27·3 per cent. as compared with an average of 71 per cent. for untreated cases during the previous four years. Confirmatory evidence of these results has been brought forward by Foulis and Barr (1937), who treated 22 cases due to infection with hæmolytic streptococci with only one death, thus giving a mortality of 1·4 per cent. as compared with 17·4 per cent. for the previous five years. The dose of sulphanilamide given was large, from 3 to 14·4 gm. daily during fever and for some days afterwards : four patients had, in addition, occasional doses of prontosil soluble. Despite the large doses toxic reactions were negligible, a somewhat unusual finding. Brown (1938) treated 39 cases of puerperal sepsis, of which 27 had local infections of the uterus, eight septicæmia and four peritonitis alone, and all were infected with hæmolytic streptococci of Type A. Prontosil and prontosil soluble were the compounds administered. Of the patients with local uterine infections, 25 recovered completely, while of those with septicæmia six recovered and of those with peritonitis all four. The evidence thus strongly suggests that despite the variability in the severity of puerperal fever, the sudden drop in mortality must of necessity be correlated with the administration of drugs of the sulphonamide group. Prontosil, it would appear, is slightly more effective in reducing the death rate than sulphanilamide. Purdie and Fry (1937) record a case in which a chronic wound infection, due to a hæmolytic streptococcus, consequent on puerperal fever three years previously, was sterilised in three weeks by 94 gm. of sulphanilamide.

Prophylaxis of Puerperal Fever. Attempts have been made to determine whether the administration of sulphonamide drugs at the time of childbirth has any effect on preventing infection during the puerperium. The first observations were made by Ley (1936), who gave prontosil to forty-two pregnant patients in whom some degree of fever, following operative interference, would naturally have been expected. Later Lacomme (1937), in France, reported the prophylactic treatment of 3,089 patients, the results being compared with the morbidity and mortality rates in 26,000 patients confined in the Baudelocque Clinic in Paris from 1926 to 1934 ; rubiazol and an allied drug were used. Puerperal infections were found to be less numerous and less severe during 1936 than in any of the previous years for which figures were available. In Great Britain, Williams (1937) used sulphanilamide prophylactically in 140 cases ; 162 being left untreated as controls. The drug was given in the third stage of labour and doses of 2·7 gm. daily were continued for a week. Analysis of the results showed little difference in the morbidity rates, but the only four cases of severe septicæmia occurred in the control series. Johnstone (1938) gave sulphonamide or proseptasine to 322 persons, of whom twenty had morbid puerperia and six local uterine infection. Among 383 women not treated prophylactically fifteen only had morbid puerperia but six of these had local uterine infections. The results of this experiment were therefore negative. MacLaughlin (1938), who gave 0·5 gm. of sulphonamide four times a day for four days, obtained a morbidity rate of 1·7 per cent. among 581 patients treated prophylactically, while among 574 women untreated prophylactically the morbidity rate was 3·5 per cent. Pigeaud and Burthiault (1937) gave 537 patients 2·5 gm. of sulphonamide for three days, beginning with the day of accouchement. There were 49 (9·1 per cent.) cases of morbidity and one death. Among 556 controls there were 63 (11·3 per cent.) cases of morbidity and no deaths. In India, Hayes and Sami (1938) gave proseptasine in doses up to 6 gm. a day. The morbidity fell from 16·94 to 11·45 per cent. In the previous year, with improvement in technique alone, the morbidity had fallen from 22·16 to 16·94 per cent.

Up to the present, therefore, mass prophylaxis of puerperal infection is hardly of practical value, since against any possible prophylactic value must be set the danger of toxic reactions. There is, however, undoubted evidence in favour of the view that sulphonamide drugs should be given to all cases in which there is undue risk of infection during the puerperium (Colebrook, 1937). One possible disadvantage of the prophylactic use of these drugs has been pointed out by Garrod (1938), who found that, by killing or damaging the causal cocci, previous sulphanilamide treatment can render the bacteriological diagnosis of any infection which may arise exceedingly difficult. It is of interest to note that Smith (1937) considered that sulphanilamide was valueless as a prophylactic in an epidemic of tonsillitis.

Streptococcal Pyelitis. A considerable number of cases of pyelitis, whether associated with pregnancy or not, are due to infection with *Bacterium coli.* The treatment of these cases is discussed on p. 415 : brief reference may, however, here be made to those cases of urinary infection in which the causal organism is a streptococcus. Cuthbert (1938), for instance, found that when sulphanilamide was administered to nine cases of urinary infection due to *Str. hæmolyticus* occurring during the puerperium, in six the urine became sterile in seven days ; in two it was sterile in fourteen days, and in one only was the bacterial content unchanged. Of eight cases infected with *Str. fæcalis*, in only two was the urine sterile in fourteen days and in the others the bacterial content was unchanged. Similar results have been obtained by other observers : fortunately, however, mandelic acid is especially useful in infections with *Str. fæcalis*.

General Septicæmia. Apart from septicæmia associated with parturition, comparatively few observations have been made on hæmolytic streptococcal septicæmia. Imhäuser (1935) and Schranz (1935), however, treated seventy-seven patients with symptoms of septicæmia, some of them following abortion, with prontosil, while Domagk (1936) reported the case of his own daughter who was attacked by a severe and rapidly spreading cellulitis as a result of running a needle into her hand. Despite surgical intervention she became stuporous, but after administration of prontosil made a complete recovery. Millar (1938) recorded recovery from septicæmia in a diabetic. Carey (1937) cured five cases of streptococcal septicæmia in children with sulphanilamide. In the treatment of septicæmia from wounds received while carrying out surgical operations or post-mortem examinations, a danger which must be regarded by surgeons and pathologists as a trade risk, drugs of the sulphonamide group should be of inestimable value. Huc (1935) has already recorded the treatment of one such case in a surgeon who developed septicæmia following an injury during an operation.

Erysipelas. In adults, erysipelas is rarely a fatal disease, so that the action of any therapeutic drug must be judged by the duration of pyrexia, the duration of spread of the rash and the general condition of the patient : in children, however, erysipelas is often fatal. The results of treatment both in children and adults have been extremely satisfactory. In the series of cases in adults reported in Germany by Anghelescu *et al.* (1936), Kramer (1936), Tonndorf (1936), Bode (1937) and Becker (1937) ; in Holland by Minkenof (1936) ; in France by Meyer-Heine and Huguenin (1936) and Bloch-Michel *et al.* (1936) ; in Great Britain by Snodgrass and Anderson (1937 [1, 2]) and Breen and Taylor (1937) ; and in the United States of America by Longcope (1938), the rash in the majority of cases ceased to spread in from twenty-four to forty-eight hours after inoculation, the temperature became normal by the fourth day and the general condition was greatly improved by the fifth day. The average duration of stay in hospital was also considerably reduced. In the earlier observations prontosil itself was given by mouth, and later sulphanilamide by the same route in from 2 to 6 gm. a day, every four hours till the temperature fell. Thereafter treatment should be given till the symptoms have completely subsided, for in certain instances (*cf.* Breen and Taylor, 1937), after a few days' administration only, relapse has occurred some days after the cessation of treatment. The only unsatisfactory results recorded in the treatment of erysipelas in the adult are those observed by Hartl (1936), who used the intravenous route, and by Angerer (1938), who found that when erysipelas was superimposed on some other condition, or *vice versâ*, no less than 28 out of his 38 cases were fatal. In children, Gmelin (1935), Püschel (1935) and Maraun (1936) reported good results with prontosil, even in infants only a few days old. Carey (1937) had only two deaths among nine patients and one of these had congenital heart disease. Very young babies were given 0·08 gm. of prontosil three or four times a day, older infants 0·15 gm. twice or thrice a day, and still older children 0·3 gm. twice or three times a day. Meyer zu Hörste (1936) recommends that in infants dilute hydrochloric acid should be given by mouth in association with each tablet.

To investigate the relative therapeutic effect of prontosil, sulphanilamide and proseptasine, Snodgrass, Anderson and Renrie (1938) studied a controlled series of 242 cases. The results are shown in the table.

Drug	No. of cases	Percentage with no spread after twenty-four hours in hospital	Percentage with no pyrexia after forty-eight hours in hospital	Percentage with no toxæmia after three days in hospital	Percentage with toxic effects
Prontosil .	58	86·2	77·6	65·5	27·5
Sulphanilamide	122	88·3	72·1	59·0	61·4
Proseptasine .	58	68·9	74·1	29·3	17·2

Although proseptasine had the smallest number of toxic reactions its therapeutic activity was less than that of either prontosil or sulphanilamide. Prontosil was less toxic than sulphanilamide, but so far as clinical results were concerned there was little difference. Prontosil was given in doses of 1 to 2 gm., 9 gm. being given per day till apparent cure and thereafter 3 gm. daily for fourteen days. Sulphanilamide was given in doses of 0·75 to 1 gm., 6 gm. being given per day till apparent cure and thereafter 3 gm. daily for fourteen days.

Cellulitis. Although there is agreement that in the treatment of streptococcal cellulitis remarkable effects may follow the administration of drugs of the sulphonamide series, it is generally believed that cellulitis does not respond as well as other streptococcal infections. Nevertheless, Goldenburg (1938) describes the cure of a case of orbital cellulitis arising from a pimple on the side of the face. At present large numbers of wounds infected with streptococci, such as were common during the war, have not been treated. Sigel (1936), however, gave prontosil soluble at an early stage to certain patients with heavily contaminated wounds and obtained primary healing.

Peritonitis. Streptococcal infections of the peritoneal cavity are by no means common, and for the most part have failed to react to sulphanilamide. Carey (1937) and Long and Bliss (1937²) report failures, but Longcope (1938) obtained cure of a premature negro child who one month after birth developed otitis media and peritonitis due to beta-hæmolytic streptococci. He recovered after repeated subcutaneous injection and one intraperitoneal injection of a solution of sulphanilamide combined with sodium lactate solution, in spite of the fact that repeated transfusions were necessary to overcome a severe hæmolytic anæmia produced apparently by sulphanilamide. Colebrook and Purdie (1937) reported the cure of two cases of streptococcal peritonitis associated with puerperal sepsis.

Arthritis. In acute infections of the joints due to hæmolytic streptococci, sulphanilamide and allied compounds may possibly be of some use. Klee and Römer (1935) reported the cure in eight weeks of a patient with acute multiple polyarthritis and endocarditis : cases of chronic arthritis were uninfluenced. Smith (1937) also recorded complete recovery in two cases of arthritis of the hip joint due to hæmolytic streptococci who were given prontosil soluble and sulphonamide.

In cases of true rheumatism, sulphonamide compounds appear to have little effect, even though hæmolytic streptococci may be isolated from the throat (Massell, 1937, and Swift *et al.*, 1938) ; in fact, in the acute stage the toxic effects of the drugs probably outweigh any beneficial effects they may have.

Gantenberg and Thimme (1935) also found that in a case of acute rheumatic fever more joints became involved during treatment with prontosil. These results are of interest in view of the uncertainty as to whether acute rheumatism is due to a streptococcus or to a virus infection.

In rheumatoid arthritis, on the other hand, a few reports have suggested improvement as a result of prontosil, but spontaneous remissions are common. Recknagel (1935), for instance, observed considerable improvement in a case of subacute rheumatoid arthritis, while Gantenberg and Thimme (1935) recorded relief, and in some instances cure, among eight cases of acute and chronic recurring arthritis, two of chronic infective arthritis and one of subacute recurring rheumatoid arthritis. In one patient with chronic infective arthritis and psoriasis treatment by prontosil produced complete disappearance of both conditions.

Tonsillitis and Infections of the Mouth and Throat. A number of authors have now described the beneficial effect of prontosil and sulphanilamide in acute tonsillitis, sometimes associated with pharyngitis or, in the cases described by Klee and Römer (1935), with lymphangitis and thrombophlebitis. Perkins (1937) found that cases treated with prontosil required a shorter period in hospital and had a shorter convalescence than untreated cases. It is realised, however, that though acute symptoms are rapidly abated, beta-hæmolytic streptococci may still persist in the throat for several weeks (Hageman, 1937; Smith, 1937). Brennerman (1937) noted that in hæmolytic streptococcal carriers the organisms disappeared as a result of sulphanilamide treatment, but reappeared from one to six days after the drug was stopped. Latent and chronic streptococcal infections of the tonsil are unaffected by sulphanilamide. In opposition to these findings Gallagher (1937), in an epidemic, concluded that adequate dosage hastened the disappearance of streptococci from the throat, but his results are open to the criticism that he did not employ sulphanilamide under the best conditions, since his routine included the administration of forced fluids. Smith (1937) treated six cases of cervical adenitis, while Fulghum (1938) reported the cure of a child suffering from Ludwig's angina resulting from streptococci entering from a wound on the chin. Cases of Ludwig's angina due to streptococci following dental operations were also reported as being cured with prontosil by Palmer (1937) and Lyth (1937). Guirdham (1937) recorded recovery with prontosil in a severe case of septic parotitis occurring in a mental patient; others have successfully treated cases of quinsy.

Scarlet Fever. Comparatively few observations have been made on long series of scarlet fever cases, probably because scarlet fever is now a relatively benign disease. The records so far published, however, suggest that the sulphonamide group of drugs is not a substitute for immune serum. Hogarth (1937), using *p*-benzylaminobenzenesulphonamide (proseptasine), could detect no difference between treated and untreated cases, while Longcope (1938) reported similar findings with sulphanilamide. Peters and Havard (1937), also using proseptasine, found that in 150 cases the duration of fever was a little longer than in 150 control cases, but the frequency of complications was slightly less in the treated series, three cases of endocarditis and three of rheumatism as against nine cases of endocarditis and ten cases of rheumatism in the controls. Long and Bliss (1936) used sulphanilamide in nineteen cases, in one of which cervical adenitis and otitis media had developed, while Massingham (1937) cured a case of septicæmia developing on the fourth day of an attack of scarlet fever. Sako, Dwan and Platou (1938) found that, though the rate of recovery from the acute toxic phase was not accelerated by the administration of sulphanilamide, the number of complications was reduced from 41 to 8 per cent. Some evidence was obtained that the drug might be of prophylactic value. Others have reported the successful treatment of cases of post-scarlatinal sinusitis. It is thus obvious that whatever its effect on uncomplicated cases, the use of sulphanilamide and its allies is clearly indicated in the treatment of the complications and sequelæ of scarlet fever.

Otitis Media and Meningitis. Records of the treatment of otitis media due to streptococcal infection have been made by Brennerman (1937), Bernstein (1937), MacIntosh *et al.* (1937). In acute cases, even when associated with signs of mastoiditis, the results have been satisfactory, and, as Hageman and Blake (1938) have shown, under sulphanilamide treatment otitis media usually subsides without discharge and mastoiditis without the necessity of operation. Perhaps the most striking success of the new drug treatment has been in cases of streptococcal meningitis, the majority of which followed otitis, although a few followed trauma or were of unexplained origin. Various estimates of the death rate of streptococcal meningitis up to 1936, when the sulphonamide group of drugs were first employed in treatment, range from 91·6 to 100 per cent. Up to the present, of thirty-nine patients from whom hæmolytic streptococci have been isolated from the cerebrospinal fluid, only 7 or 8 per cent. have died following treatment with prontosil or sulphanilamide. In some cases the patients (*cf.* Weinberg *et al.*, 1937, and Vitenson and Konstam, 1937) were almost moribund and the response to treatment was little short of miraculous. The cerebrospinal fluid has in certain instances become sterile in twenty-four hours, but in others not till seven days from the beginning of treatment (Carey, 1937). One infection by anaerobic hæmolytic streptococci has been successfully cured by the administration of sulphanilamide by mouth (Smith *et al.*, 1938). Although streptococcal meningitis is undoubtedly amenable to treatment with sulphonamide drugs, there is still much uncertainty as to the best method of administration. Thus Neal and Appelbaum (1938) used the intrathecal route: thirteen of their seventeen cases recovered. Flake and Carey (1937), whose four cases all recovered, gave sulphanilamide intrathecally as well as subcutaneously and by mouth. Trachsler *et al.* (1937), with four recoveries out of seven cases, and Weinberg *et al.* (1937), with two successful cases, gave prontosil intramuscularly and sulphanilamide orally. Apparently 0·8 per cent. sulphanilamide in saline injected intrathecally causes no untoward reaction, but prontosil given by this route may cause fever and intense cellular reaction (Millett, 1937, and Schwentker *et al.*, 1937). In view of the rapidity with which drugs of the sulphonamide series reach the cerebrospinal fluid after oral administration, the value or necessity of the intrathecal route is highly questionable. Lovelace (1938) has recently reported the successful treatment of a case of meningitis due to *Str. viridans*.

Pneumonia and Empyema. In streptococcal pneumonia, sometimes secondary to influenza, sulphanilamide and prontosil have, it is claimed, occasionally brought about rapid improvement. Scheurer (1936), for instance, noted rapid defervescence and improvement in the pulmonary condition in a case treated with prontosil. Herliuson (1937), in a series of 300 patients suffering from influenza in 1935–1936, noted 22·7 per cent. of complications, including pneumonia, empyema and meningitis ; seven patients died. In the next series of cases treatment with prontosil was begun at an early stage and the incidence of complications was only 3·1 per cent. with no deaths. Further observations made during one and the same epidemic are obviously necessary before the significance of these figures can be appreciated. Fleming (1937) described a case of empyema, treated with sulphanilamide by mouth with apparent improvement, while Tiling (1936) and Brown (1937) have treated three cases with injections into the pleural cavity of 5 c.c. of prontosil soluble (2·5 per cent.). One of the patients also received prontosil by mouth. The injection of sulphonamide compounds into the pleural cavity is, however, not without danger, for in rabbits with experimentally produced streptococcal empyema Nicholson (1938) found that those animals in which prontosil soluble was injected into the pleural cavity died more rapidly than the controls. Tixier and Eck (1935) treated empyema in children by giving prontosil by mouth, while Douthwaite (1937) gave an adult the same drug orally and by intramuscular injection with good results.

Other Streptococcal Conditions. Many other conditions due to streptococci have from time to time been treated with sulphonamide drugs with some apparent success, such as impetigo contagiosa, pemphigus vulgaris, lupus erythematosus (Linser, 1938), subacute bacterial endocarditis and infections of the genito-urinary tract. In those infections caused by *Str. viridans* or *Str. fæcalis* the beneficial effects of the therapy have been very uncertain.

References

ANGERER, H., 1938, Zur Beurteilung und Behandlung leichter und schwerer Erysipele. *Med. Klinik.*, XXXVI., 175.

ANGHELESCU, V., CRIVETZ, D., PASCAL, I., and LA ZARESU, V., 1936, Behandlung des Erysipels mit Prontosil bei gleichzeitiger Erkrankung der Nieren. *Dtsch. med. Wschr.*, LXII., 1639.

BAYER PRODUCTS LTD., 1938, Prontosil : a survey of the new chemotherapy. London.

BECKER, W., 1937, Die Behandlung des Erysipels mit Prontosil. *Derm. Wschr.*, CIV., 221.

BERNSTEIN, S. S., 1937, Report on use of sulfanilamide at Children's Hospital of Michigan. *J. Pediat.*, XI., 198.

BLISS, E. A., and LONG, P. H., 1937, Failure of para-aminobenzenesulfonamide therapy in urinary tract infections due to Group D (Lancefield) beta-hemolytic streptococci. *New Engl. J. Med.*, CCXVII., 18.

BLOCH-MICHEL, H., CONTE, M., and DURIL, P., 1936, L'emploi des dérivés sulfamidés non azotiques dans le traitement de l'erysipèle. *Pr. méd.*, 1583.

BODE, H. G., 1937, Die Behandlung des Erysipels. *Z. ärztl. Fortbild.*, 587.

BOSSE, O. A., 1936, Die Therapie der Streptokokkenerkrankungen mit "Prontosil." *Fortschr. Ther.*, XII., 540.

BREEN, J. E., and TAYLOR, I., 1937, Erysipelas treated with prontosil. *Lancet, i.*, 1334.

BRENNERMAN, J., 1937, Report on sulfanilamide from Children's Memorial Hospital of Chicago. *J. Pediat.*, XI., 238.

BROWN, D. B., 1938, Treatment of puerperal sepsis by prontosil and allied compounds. *J. Obstet. Gynæc. Brit. Emp.*, XLV., 495.

BROWN, J. L., 1937, Prontosil by the intrapleural route : two cases of streptococcal empyema. *Brit. med. J.*, *i.*, 157.

BUTTLE, G. A. H., GRAY, W. H., and STEPHENSON, D., 1936, Protection of mice against streptococcal and other infections by *p*-aminobenzenesulphonamide and related substances. *Lancet*, *i.*, 1286.

CAREY, B. W., 1937, The use of para-aminobenzenesulfonamide and its derivatives in the treatment of infections due to the β-streptococcus hemolyticus, the meningococcus and the gonococcus. *J. Pediat.*, XI., 202.

COLEBROOK, L., 1937, Prophylactic treatment in obstetrics by means of prontosil and sulphanilamide. *Lancet*, *ii.*, 286.

COLEBROOK, L., KENNY, M., and Staff of Queen Charlotte's Hospital, 1936[1], Treatment of human puerperal infections and of experimental infections in mice with prontosil. *Ibid.*, *i.*, 1279.

COLEBROOK, L., KENNY, M., and Staff of Queen Charlotte's Hospital, 1936[2], Treatment of prontosil infections due to hæmolytic streptococci. *Ibid.*, *ii.*, 1319.

COLEBROOK, L., and PURDIE, A. W., 1937, Treatment of 106 cases of puerperal fever by sulphanilamide (streptocide). *Ibid.*, *ii.*, 1237.

CUTHBERT, J. C., 1938, The treatment of urinary infections in the puerperium. *Ibid.*, *ii.*, 728.

DOMAGK, G., 1936, Chemotherapie der Streptokokkeninfektionen. *Klin.*, *Wschr.*, XV., 1585.

DOUTHWAITE, A. H., 1937, The use of antistreptococcal preparations in general practice. *Practitioner*, CXXXIX., 661.

FLAKE, C. G., and CAREY, B. W., 1937, Para-aminobenzenesulfonamide and its derivatives in the treatment of beta hemolytic streptococcus infections of the middle ear and mastoid. *New Engl. J. Med.*, CCXVII., 1033.

FLEMING, J. B., 1937, Streptocide for streptococcal empyema. *Brit. med. J.*, *i.*, 1281.

FOULIS, M. A., and BARR, J. B., 1937, Prontosil album in puerperal sepsis. *Ibid.*, *i.*, 445.

FULGHUM, J. E., 1938, Sulfanilamide treatment of Ludwig's angina. *Nav. med. Bull., Wash.*, XXXVI., 58.

GALLAGHER, J. R., 1937, Observations on therapeutic value of sulfanilamide in beta-hemolytic streptococcus pharyngitis. *Amer. J. med. Sci.*, CXCIV., 830.

GANTENBERG, R., and THIMME, B., 1935, Über Erfahrungen mit Prontosil. *Med. Welt.*, IX., 1009.

GARROD, L. P., 1938, The chemotherapy of bacterial infections. *Lancet*, *i.*, 1125 and 1178.

GIBBERD, G. F., 1937, Prontosil and similar compounds in the treatment of puerperal hæmolytic streptococcus infections. *Brit. med. J.*, *ii.*, 695.

GMELIN, L., 1935, Zur Chemotherapie des Erysipels im Kindesalter. *Münch. med. Wschr.*, LXXXII., 221.

GOLDENBURG, M., 1938, Sulfanilamide in ophthalmology. *Amer. J. Ophthal.* XXI., 54.

GROSS, P., COOPER, F. B., and PEEBLES, L. M., 1937, Effect of splenectomy on the therapeutic action of *p*-aminobenzenesulfonamide on mice infected with hemolytic streptococcus. *Proc. Soc. exp. Biol., N.Y.*, XXXVI., 311.

GUIRDHAM, A., 1937, Prontosil in septic parotitis. *Brit. med. J.*, *i.*, 997.

HAGEMAN, P. O., 1937, Clinical experience in use of sulfanilamide at New Haven Hospital. *J. Pediat.*, XI., 195.

HAGEMAN, P. O., and BLAKE, F. G., 1938, Clinical experience with sulfanilamide in treatment of beta hemolytic streptococcus infections. *Amer. J. med. Sci.*, CXCV., 163.

HARTL, K., 1936, Die Behandlung des Erysipels der Erwachsenen mit Prontosil. *Dtsch. med. Wschr.*, LXII., 1641.

HAYES, S. N., and SAMI, A., 1938, A clinical investigation on the value of proseptasine as a prophylactic in puerperal infection. *Ind. med. Gaz.*, LXXIII., 328.

HERLIUSON, H., 1937, Traitement préventif et curatif des complications grippales par la sulfamido-chrysoïdine. Thèse, Paris.

HOGARTH, J. C., 1937, Para-benzylaminobenzenesulphonamide in the treatment of scarlet fever. *Brit. med. J.*, *ii.*, 1160.

HUC, H., 1935, Discussion on paper by Tixier and Eck. *Bull. Soc. Pediat., Paris*, XXXIII., 501.

IMHÄUSER, K., 1935, Über die Behandlung septischer Erkrankungen mit Prontosil. *Med. Klinik.*, IX., 282.

JOHNSTONE, R. W., 1938, Prophylactic use of sulphonamide preparations in obstetric practice. *Brit. med. J.*, *i.*, 562.

KLEE, P., and RÖMER, H., 1935, Prontosil bei Streptokokkenerkrankungen. *Dtsch. med. Wschr.*, LXI., 253.

KRAMER, W., 1936, Ueber Erfahrungen bei der Erysipelbehandlung mit Prontosil. *Münch. med. Wschr.*, LXXXIII., 608.

LACOMME, M., 1937, Nouveaux résultats de l'essai de prophylaxie des infections puerpérales institué à la clinique Baudelocque. Considérations sur l'interprétation de ces résultats. *Bull. Soc. Obstét. Gynéc. Paris*, XXVI., 459.

LEY, L., 1936, Zur Behandlung des Puerperalfiebers mit Prontosil. *Münch. med. Wschr.*, LXXXIII., 1092.

LINSER, K., 1938, Heilung von Lupus erythematodes durch Prontosilbehandlung. *Derm. Wschr.*, XII., 342.

LONG, P. H., and BLISS, E. A., 1936, quoted in *Sci. News Lett.*, XXX., 339.

LONG, P. H., and BLISS, E. A., 1937[1], The clinical use of sulphanilamide and its derivatives in the treatment of infectious diseases. *Ann. intern. Med.* XI., 575.

LONG, P. H., and BLISS, E. A., 1937 [2], para-Aminobenzenesulfonamide and its derivatives. Clinical observations on their use in the treatment of infections due to beta hemolytic streptococci. *Arch. Surg., Chicago*, **XXXIV.**, 351.

LONGCOPE, W. T., 1938, Problems relating to the invasive properties of hemolytic streptococci and their control by sulphanilamide. *Amer. J. med. Sci.*, **CXCV.**, 577.

LOVELACE, J. W., 1938, Non-hemolytic streptococcic meningitis : report of a case successfully treated with sulfanilamide and prontosil. *J. lab. clin. Med.*, **XXIII.**, 482.

LYTH, H., 1937, Ludwig's angina treated with prontosil. *Brit. med. J.*, *i.*, 584.

MACINTOSH, R., WILCOX, D. A., and WRIGHT, F. H., 1937, Results of sulfanilamide treatment at Babies Hospital, New York City : survey of 58 cases observed prior to June 10th, 1937. *J. Pediat.*, **XI.**, 167.

MACLAUGHLIN, J. H., 1938, Sulphonamide for prophylaxis. *Brit. med. J.*, *i.*, 757.

MARAUN, L., 1936, Zur Prontosilbehandlung des Erysipels und der Pyurie. *Kinderärztl. Prax.*, **X.**, 445.

MASSELL, B. F., 1937, Studies on the use of prontylin in rheumatic fever. *New Engl. J Med.*, **CCXVI.**, 487.

MASSINGHAM, R., 1937, The use and abuse of drugs in the acute specific fevers. *Practitioner*, **CXXXVIII.**, 465.

MEUNIER, P., and NITTI, F., 1938, L'action protectrice du *p*-aminobenzenesulfamide (1162F) dans les infections streptococciques expérimentales chez le rat carencé en vitamine A. *C. R. Soc. Biol., Paris*, **CXXVII.**, 688.

MEYER-HEINE, A., and HUGUENIN, P., 1936, Traitement de l'erysipèle par le chlorhydrate de sulfamidochrysoidine. *Pr. méd.*, **XLIV.**, 454.

MEYER ZU HÖRSTE, G., 1936, Zur Prontosilbehandlung. *Klin. Wschr.*, **XV.**, 1602.

MILLAR, J. H. D., 1938, Recovery from hæmolytic streptococcal septicæmia in patient with diabetes. *Brit. med. J.*, *ii.*, 404.

MILLETT, J., 1937, Hemolytic streptococcus meningitis : report of a case treated with sulphanilamide and prontosil soluble intramuscularly and intrathecally with recovery. *New Engl. J. Med.*, **CCXVII.**, 556.

MINKENOF, J. E., 1936, De Behandeling van Erysipelas mit Prontosil. *Ned. Tijdschr. Geneesk.*, **IV.**, 5197.

MUETHER, R. O., and KINSELLA, R. A., 1938, Sulfanilamide in the treatment of experimental endocarditis. *J. Amer. med. Ass.*, **CVIII.**, 603.

NEAL, J. B., and APPELBAUM, E., 1938, Experience with sulfanilamide in meningitis. *Amer. J. med. Sci.*, **CXCV.**, 163.

NICHOLSON, W. F., 1938, Treatment of streptococcal empyema with intrapleural sulphanilamide. *Brit. med. J.*, *i.*, 115.

PALMER, W. H., 1937, Ludwig's angina treated with prontosil. *Ibid.*, *i.*, 472.

PERKINS, H. N., 1937, Treatment of acute follicular tonsillitis by prontosil. *J. roy. Army med. Corps.*, **IV.**, 267.

PETERS, B. A., and HAVARD, R. V., 1937, Chemotherapy of streptococcal infections with *p*-benzylaminobenzenesulphonamide. *Lancet*, *i.*, 1273.

PIGEAUD, H., and BURTHIAULT, R., 1937, Essai de prophylaxie des infections puerpérales " post partum " à l'aide d'un médicament à action antistreptococcique. *Lyon. méd.*, **CLX.**, 691.

PURDIE, A. W., and FRY, R. M., 1937, Chronic hæmolytic streptococcal infection treated with *p*-aminobenzenesulphonamide. *Lancet*, *ii.*, 18.

PÜSCHEL, E., 1935, Erysipelbehandlung im Säuglingsalter. *Fortschr. Ther.*, **XI.**, 96.

Recknagel, K., 1935, Erfahrungen mit Prontosil. *Münch. med. Wschr.*, LXXXII., 704.

Sako, W., Dwan, P. F., and Platou, E. S., 1938, Sulfanilamide and serum in the treatment and prophylaxis of scarlet fever. *J. Amer. med. Ass.*, CXI., 995.

Scheurer, O., 1936, Unsere Erfahrungen mit Prontosil. *Med. Klinik.*, XXII., 739.

Schranz, H., 1935, Zur Sepsisbehandlung mit Prontosil. *Münch. med Wschr.*, LXXXII., 419.

Schwentker, F. F., Clason, F. P., Morgan, W. A., Lindsay, J. W., and Long, P. H., 1937, Use of para-amino-benzene-sulphonamide or its derivatives in treatment of beta hæmolytic streptococcal meningitis. *Johns Hopk. Hosp. Bull.*, LX., 297.

Seastone, C. V., 1937, The effect of sulfanilamide (para-aminobenzene-sulfonamide) on Group "C" hemolytic streptococcus infections. *J. Immunol.*, XXXIII., 407.

Sigel, O., 1936, "Prontosil soluble" bei chirurgischen Erkrankungen. *Fortschr. Ther.*, XII., 229.

Smith, A., 1937, Chemotherapy of streptococcal infections. *Lancet, ii.*, 1064.

Smith, F. W., Mayer, K. F., Agerty, H. A., Fowler, R. H., and Layton, W. C., 1938, Anaerobic beta hemolytic streptococcus meningitis of otitic origin treated with sulfanilamide and culminating in complete recovery. *J. Amer. med. Ass.*, CX., 887.

Snodgrass, W. R., and Anderson, T., 1937 [1], Prontosil in the treatment of erysipelas : controlled series of 312 cases. *Brit. med. J.*, *ii.*, 101.

Snodgrass, W. R., and Anderson, T., 1937 [2], Sulphanilamide in the treatment of erysipelas : a controlled series of 270 cases. *Ibid.*, *ii.*, 1156.

Snodgrass, W. R., Anderson, T., and Renrie, J. L., 1938, Sulphamido-chrysoidine, sulphanilamide and benzylsulphanilamide in the treatment of erysipelas. A controlled series of cases. *Ibid.*, *ii.*, 399.

Swift, H. F., Moen, J. K., and Hirst, G. K., 1938, The action of sulfanilamide in rheumatic fever. *J. Amer. med. Ass.*, CX., 426.

Tiling, B., 1936, Empyem-Behandlung mit Prontosil. *Münch. med. Wschr.*, XXIII., 952.

Tixier, L., and Eck, M., 1935, Trois cas de pleurésies purulent streptocoques guéris par l'ingestion du chlorhydrate de sulfamido-chrysoïdin (rubiazol). *Bull. Soc. Pédiat.*, *Paris*, XXXIII., 493.

Tonndorf, H., 1936, Eine lückenlose Reihe von Erysipelheilungen durch Prontosil. *Med. Klinik.*, XXXII., 1307.

Trachsler, W. H., Frauenberger, G. S., Wagner, C., and Mitchell, A. G., 1937, Streptococcic meningitis with special emphasis on sulfanilamide treatment. *J. Pediat.*, XI., 248.

Vitenson, I., and Konstam, G., 1937, Case of streptococcal meningitis treated with prontosil. *Lancet*, *i.*, 870.

Weinberg, M. H., Mellon, R. R., and Shinn, L. E., 1937, Two cases of streptococcic meningitis treated successfully with sulfanilamide and prontosil. *J. Amer. med. Ass.*, CVIII., 1948.

Williams, B., 1937, Prophylactic treatment in obstetrics by means of prontosil and sulphanilamide. *Lancet*, *ii.*, 343.

(c) Streptococcal Infections in Domestic Animals

Domestic animals, such as the cow, horse and dog, all suffer from hæmolytic streptococcal infections, not, however, necessarily belonging to Group A. Up to the present very few observations have been reported on the effect of the sulphonamide group of drugs on these infections, and unfortunately in the majority of those that have been recorded no attempt has been made to find out the causal organisms, much less to obtain evidence of their type. Whicher and Lambert Smith (1937) used prontosil in the successful treatment of puerperal infections in dogs and in a septic wound following an operation for uterine prolapse. A chronic suppurative nasal discharge in a cat was also cured. Haselden (1938) reported the treatment in dogs of a mammary abscess, puerperal sepsis and pyelonephritis with prontosil, while the same drug was employed with equal success in puerperal sepsis in a mare and in a septic wound of the foot in a cat. In bovine mastitis a beginning has been made in treatment with sulphanilamide by Allott (1937) and Bauer and Gunderson (1938), who found that in three animals the number of organisms in the milk decreased while in a fourth streptococci vanished from the milk so long as sulphanilamide was administered. The results are of sufficient promise to warrant controlled experiments to determine whether sulphanilamide produces better results than udder infusions with such drugs as acriflavine or rivanol (*cf.* Wolf and Leskien, 1937 ; Stableforth and Scorgie, 1938).

Bryant (1938) successfully treated a case of mastitis in a sow.

Dogs infected with canine distemper virus frequently die from pneumonia caused mainly by hæmolytic streptococci of Type G : the death rate can be considerably reduced by sulphanilamide in daily doses of 0·5 gm. per kilogram of body weight (MacIntyre and Buttle, 1938), or by intravenous injections of rubiazol (Groulade, 1937).

Feline distemper (infectious enteritis) is also complicated in a high percentage of cases by peritonitis caused by hæmolytic streptococci and the mortality is usually 100 per cent. This mortality can also be decreased by sulphanilamide.

Prontosil soluble was found by Habs and Röper (1937) to be of little or no value in the treatment of streptococcal lymphadenitis in guinea-pigs.

Davesne and Brunschwick (1937) were successful in treating prophylactically a septicæmia of new-born lambs due to a streptococcus. Prontosil was given in a dose of 0·2 gm. on the day of birth and on the following day. In horses, cases of streptococcal pneumonia have been successfully treated by sulphanilamide.

For the larger animals, such as horses and cows, doses of 1 gm. per 10 lb. of body-weight daily by mouth are well tolerated (Stableforth, 1938). In cattle, diarrhœa and incoordination of gait are sometimes seen, while sulphæmoglobinæmia has been noted in the horse and dog.

The chemotherapeutic treatment of streptococcal infections in domestic animals requires much fuller investigation, for though Group A hæmolytic streptococci are responsible only for a small number of cases of acute bovine mastitis, Group C streptococci also react to the sulphonamide group of drugs, and these organisms cause strangles in horses, metritis in mares, certain cases of acute mastitis and possibly joint ill.

References

ALLOTT, A. J., 1937, The treatment of bovine mastitis with sulfanilamide. A preliminary report. *J. Amer. vet. med. Ass.*, XCI., 588.

BAUER, H., and GUNDERSON, M. F., 1938, The effect of sulfanilamide upon the streptococci in the udder of mastitis cows and a method for its estimation in milk. *J. Bact.*, XXXV., 66.

BRYANT, J. B., 1938, Mastitis in sow relieved by sulfanilamide. *J. Amer. vet. med. Ass.*, XCIII., 117.

DAVESNE, J., and BRUNSCHWICK, M., 1937, Traitement préventif de la septicémie des agneaux par la sulfamido-chrysoïdine. *C. R. Soc. Biol., Paris*, CXXV., 335.

GROULADE, 1937, Les colorants azoïques dans le traitement de la maladie de Carré. *Bull. Acad. vét. de France*, X., 345.

HABS, H., and RÖPER, E., 1937, Chemotherapeutische Versuche mit "Prontosil" bei der epizootischen Streptokokkenlymphadenitis der Meerschweinchen. *Z. Immunforsch.*, XCI., 360.

HASELDEN, C. S., 1938, Notes on the use of prontosil in veterinary practice. *Vet. Rec.*, L., 439.

MACINTYRE, A. B., and BUTTLE, G. A. H., 1938. (Unpublished observations.)

STABLEFORTH, A. W., 1938, Streptococcus infections of animals and their treatment. *Vet. Rec.*, L., 1203.

STABLEFORTH, A. W., and SCORGIE, N. J., 1938, Entozon and acriflavine for the treatment of contagious bovine mastitis. *Vet. Rec.*, L., 663.

WHICHER, A., and SMITH, H. L., 1937, Some observations on the use of prontosil in canine practice. *Vet. Rec.*, XLIX., 452.

WOLF, C. H., and LESKIEN, I., 1937, Weitere Prüfungen verschiedener zum Zwecke der Galtbehandlung empfohlener Heilmittel. III. Mitteilung. Trypaflavin nach Steck. *Dtsch. tierärztl. Wschr.*, XLV., 292.

OTHER COMPOUNDS IN THE TREATMENT OF STREPTOCOCCAL INFECTIONS

Sulphanilamide Derivatives

In view of the successful results obtained with prontosil and sulphanilamide in the treatment of streptococcal infections, very intensive study has been devoted to other related compounds, using as test objects mice infected with hæmolytic streptococci. Domagk (1935) found that, in addition to prontosil, the disodium salt of 4′-sulphonamidophenylazo-1-hydroxy-7-acetylaminonaphthalene-3 : 6-disulphonic acid (prontosil soluble) was active, not only against streptococcus, but also against staphylococcus and certain strains of the pneumococcus Type III in mice. Some years previously Heidelberger and Jacobs (1919) had prepared a representative of the same chemical group, 4-sulphamidobenzeneazohydrocupreine, which was bactericidal *in vitro*. Levaditi and Vaisman (1935) also studied the sodium salt of 4-sulphamidobenzeneazo-3 : 5-diaminobenzoic acid. All these substances are coloured and possess very well developed tinctorial properties.

Following on the demonstration of the anti-streptococcal activity of *p*-aminobenzenesulphonamide, Goissedet *et al.* (1936) showed that 4-benzylideneaminobenzenesulphonamide, *o*-hydroxy-4-benzylideneaminobenzenesulphonamide, *p*-hydroxy-4-benzylideneaminobenzenesulphonamide, dihydroxybenzylideneaminobenzenesulphonamide and 2 : 4 : 6-trihydroxybenzylideneaminobenzenesulphonamide were all active in curing streptococcal infections in mice. Hydrogenation of the benzylidene group led to the formation of benzylamino derivatives, but all these preparations were inactive against streptococci, with the exception of *p*-benzylaminobenzenesulphonamide. This compound (proseptasine) was shown to be of low toxicty for mice and to have a well marked curative action in streptococcal infections. These results were confirmed by Halpern and Mayer (1936) and Mayer (1937), who found that proseptasine had a chemotherapeutic index in streptococcal infections in mice of 1 : 40, as compared with 1 : 10 for sulphanilamide and 1 : 4 for prontosil. In Great Britain, Whitby (1937) reported that in doses of 50 mgm. orally for 20 gm. mice proseptasine is highly effective in bringing about a cure of streptococcal infections. Although mice can be given orally 20 gm. per kilogram of body weight without showing symptoms, the majority of observers find it less efficient in mice than sulphanilamide. The following compounds also had some action in streptococcal infections: disodium *p*-(γ-phenylpropylamino)-benzenesulphonamide *a*γ-disulphonate (soluseptasine), 4 : 4′-diaminobenzenesulphonanilide tartrate and 4 : 3′-diaminobenzenesulphonanilide. Soluseptasine, which is slightly toxic in doses of 200 mgm. for a 20 gm. mouse, is effective in doses of 10 mgm. by mouth, as is 4 : 4′-diaminobenzenesulphonanilide tartrate. 4 : 3′-Diaminobenzenesulphonanilide, which is non-toxic by mouth in doses of 100–160 mgm., is active in doses of 20 mgm. Gray, Buttle and Stephenson (1937), however, found proseptasine inferior to sulphaniliamide in the treatment of streptococcal infections in mice, but Cooper, Gross and Lewis (1938) confirmed the therapeutic activity of 4 : 4′-diaminobenzenesulphonanilide against certain strains of hæmolytic streptococci in mice in doses by mouth of 10 mgm. Although slightly more toxic it proved as good as, or more efficient than, sulphanilamide. No demonstrable hepatic or renal lesions were produced. Suspensions in gum acacia were unstable.

The results obtained by Buttle, Gray and Stephenson (1936) with a number of substances related to *p*-aminobenzenesulphonamide are shown in the table on page 366, in relation to Formulæ I and II. They may be summarised as follows :—

(*a*) **Substances in which the Amino Group —NH_2 in Position 1, Formula I, is modified or replaced.** Replacement of the —NH_2 group by —NH—CO—CH_3, —$N(CH_3)$—CO—CH_3, and —CH_3 produces compounds with much less activity. A series of azo compounds in which —NH_2 is replaced by —N = NR, where R is the dihydrocupreine, dihydrocupreidine, *apo*quinine, or *isoapo*quinine residue, or = N—N = R′, where R′ is the tropinone residue, all proved only slightly active, as did an azo compound in which R was the protein of mouse serum. Replacement of the—NH_2 by —H, —OH, or —Cl, produced inactive compounds.

(*b*) **Substances in which the Sulphonamide Group in Position 4, Formula I, was modified.** Substitution of —C_6H_5 for one of the hydrogen atoms of the sulphonamide group produced a compound (*p*-aminobenzenesulphonanilide) as active as *p*-aminobenzenesulphonamide: the acetyl derivative was less active. Sulphanilic acid, containing the group —SO_2—OH in place of —SO_2—NH_2, was only one-twentieth as active. Modification of either group attached to the benzene nucleus diminished or destroyed the activity with the exception of the anilide: *m*- and *p*-aminoacetanilide and *o*-, *m*- and *p*-nitroacetanilide were highly toxic and showed no action in tolerated doses.

(*c*) **Introduction of Additional Substituents into the Benzene Nucleus, Formula I.** Two bromine atoms in the 2 and 6 positions gave an inactive compound. 1-Aminobenzene-2 : 4 : 6-trisulphonamide was also inactive.

(*d*) ***p*-Toluenesulphonamide Derivatives, Formula I.** *p*-Toluenesulphonethylamide was less active than *p*-toluenesulphonamide and the corresponding diethyl-compound was inactive.

(*e*) **Sulphonic Acids, Formula I.** *m*-Aminobenzenesulphonic acid was slightly active. Sulphanilic acid has been mentioned above. Phenylhydrazine-*p*-sulphonic acid had a slight activity. Benzenesulphonic acid and 4-nitro-1-aminobenzene-2-sulphonic acid were both inactive. The presence of the basic group (—NH_2) attached to the benzene ring appears to be as important as that of the sulphonamide group alone.

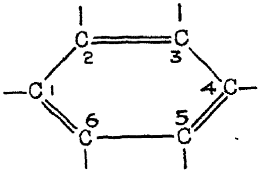
(*f*) **Naphthalene Derivatives, Formula II.** *α*- and *β*-Naphthalenesulphonamides were both inactive ; *α*-acetylaminonaphthalene-4-sulphonamide was active but not quite as good as the corresponding benzene derivatives. *α*-Naphthylamine-4-sulphonic acid had the same degree of activity as sulphanilic acid, the benzene analogue: *α*-naphthylamine-5-sulphonic acid and *β*-naphthylamine-1-sulphonic acid, on the other hand, were quite inactive.

The finding that sulphanilic acid has a distinct though smaller activity in mice than sulphanilamide is of considerable interest, since it is hardly to be supposed that this would be changed into the amide in the body.

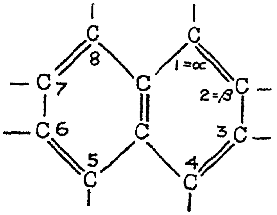
Fourneau *et al.* (1936 and 1937 [1]) have found that changes in the structure of sulphanilamide result in most cases in a decrease in streptococcicidal activity. Their observations may be summarised as follows :—

CHEMOTHERAPEUTIC ACTION OF SUBSTANCES RELATED TO *p*-AMINOBENZENESULPHONAMIDE ON STREPTOCOCCAL INFECTION IN MICE (Buttle, Gray and Stephenson, 1936)

Compound	Orientation in Formula I	Melting point	Therapeutic activity
p-Aminobenzenesulphonamide .	$NH_2 : SO_2—NH_2 = 1 : 4$	165°	
p-Acetylaminobenzenesulphonamide	$NH—CO—CH_3 : SO_2—NH_2 = 1 : 4$	218°	
N-Methyl-*p*-acetylaminobenzenesulphonamide . . .	$N(CH_3)—CO—CH_3 : SO_2—NH_2 = 1 : 4$	153°	
p-Toluenesulphonamide . .	$CH_3 : SO_2—NH_2 = 1 : 4$	144°	
p-Sulphonamidobenzeneazodihydrocupreine . . .	$C_{23}O_2N_2 : SO_2—NH_2 = 1 : 4$		
Tropinone *bis*-sulphonamidophenylhydrazone acetate .	$N_2—C_{16}H_{21}O_5N_4S : SO_2—NH_2 = 1 : 4$	210°	
p-Aminobenzenesulphonanilide .	$NH_2 : SO_2—NH—C_6H_5 = 1 : 4$	198°	
p-Acetylaminobenzenesulphonanilide	$NH—CO—CH_3 : SO_2—NH—C_6H_5 = 1 : 4$	214°	
Sulphanilic acid . . .	$NH_2 : SO_2—OH = 1 : 4$		
m-Aminobenzenesulphonic acid .	$NH_2 : SO_2—OH = 1 : 3$		
	Orientation in Formula II		
α-Acetylaminonaphthalene-4-sulphonamide . .	$NH—CO—CH_3 : SO_2—NH_2 = 1 : 4$	247°	
Sodium α-naphthylamine-4-sulphonate . .	$NH_2 : SO_2—ONa = 1 : 4$		



Formula I



Formula II

(*a*) **Influence of the Relative Position of the Sulphonamide and Amino Groups.** Of the three isomers the ortho- and meta-sulphonamide were devoid of action.

(*b*) **Replacement of the Sulphonamide Group by Various Other Groups.** Relacement of SO_2NH_2 by NH_2,CN,SO_3H,AsO_3H_2,$CONH_2$ produced inactive compounds.

(*c*) **Substitutions in the Sulphonamide Group.** Alkylation of the sulphonamide group does not suppress activity, but, at least in the higher members of the series, decreases it in proportion to the weight of the substituted alkyl radical.

(*d*) **Replacement of the Amino-Group by Another Radical.** The *p*-hydroxy derivative is slightly active ; the *p*-methyl derivative inactive.

(*e*) **Derivatives of Substitution on the Amino-Group.** When the amino-group of *p*-aminobenzenesulphonamide is replaced by the groups HCONH, CH_3CONH, C_2H_5OCONH, NH_2CH_2CONH, NH_2COCH_2NH the resulting compounds are active.

(*f*) **Azo-compounds with Substituents in the Sulphur-free Nucleus.** A number of mono-, di- or trihydroxy derivatives of the second nucleus are active, such as 4′-hydroxy-2′ : 4′dihydroxy, 2′ : 4′ : 6′-trihydroxybenzylazo-1′-4′-benzenesulphonamide, and 4′ : 6′-dihydroxyalkyl-5′-phenylazo-1′-4′-benzenesulphonamides.

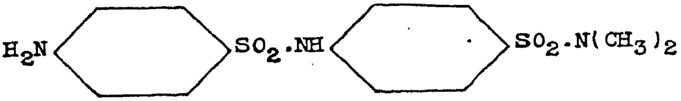
(*g*) **Addition of a Third Substituent in the Benzene Nucleus.** This is followed by a decrease in activity. A number of inactive derivatives were also tested, including quinquevalent arsonic acids, quinoline, naphthalene, phenoxazine and urotropine derivatives.

Gray, Buttle and Stephenson (1937) obtained certain compounds which were as active as *p*-aminobenzenesulphonamide and occasionally less toxic. This was the case noticeably with 2′ - pyrrolidone - 5′ - carboxy - 4 - aminobenzenesulphonamide, *p*-aminobenzenesulphonyl-*p*-sulphonamidophenylamide hydrochloride, *p*′-methoxybenzylideneaminobenzenesulphonamide and *p*′ - dimethylaminobenzylidene - *p* - aminobenzenesulphonamide. Prontosil, prontosil soluble and *p*-sulphonamidoglucoseanil were found to be of about the same therapeutic activity as *p*-aminobenzenesulphonamide.

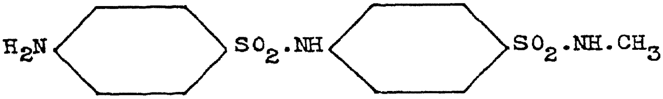
Domagk (1937) also investigated *p*-aminobenzenesulphonyl-*p*′-aminobenzenesulphonamide (Diseptal C, DB 32, disulphanilamide), which had been synthesised by Klarer and Mietzsch, and found it perhaps slightly superior to sulphanilamide in its action on streptococcal infections in mice : similar results were obtained by Rosenthal, Bauer and Branham (1937). Disulphanilamide is relatively non-toxic to mice, and Barlow (1937) has shown that mice receiving up to 40 gm. per kilogram of body weight showed no toxic effects. Feinstone *et al.* (1938), however, believe from estimations on the blood that there is no increase in absorption of the compound after doses in excess of 1 gm. per kilogram. Raiziss *et al.* (1938) found its therapeutic action greater when injected subcutaneously than when given orally.

In addition to the non-methylated compound, Domagk (1937) investigated the dimethylamide and the monomethylamide. The former, under the name of " Uleron " or " Diseptal A," has been used in the treatment of gonorrhœa (*cf.* p. 403), while Domagk himself believed that it possessed advantages over sulphanilamide in the treatment of staphylococcal infections. Buttle *et al.* (1938) were, however, unable to confirm this finding (*cf.* p. 410).

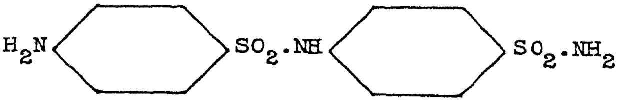
Uleron, Diseptal A = DB 90



Diseptal B = DB 87

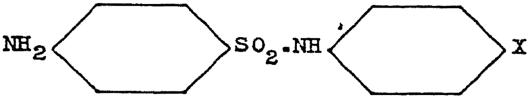


Diseptal C = DB 32



All these compounds are, in comparison with sulphanilamide, more or less equally effective in streptococcal infections in mice. In the case of uleron, it has been found that the only product of excretion in rabbits is its acetyl compound, which is present in large quantities in the urine. Rosenthal *et al.* (1937) and Bauer and

Rosenthal (1938), following on the observations of Buttle, Gray and Stephenson (1936), prepared substances by the substitution of groups in the *para*-position of the anilide of sulphanilamide.



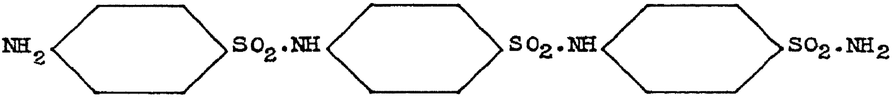
When X was COOH, *p*-aminobenzenesulphonyl-*p*-aminobenzoic acid resulted, the sodium salt of which was freely soluble in water. Though of low toxicity it had little or no action in streptococcal infections.

When X was NO_2, sulphanil-*p*-nitroanilide resulted: this compound was two or three times as active by mouth as sulphanilamide in streptococcal infections but twice as toxic.

When X was NH_2, sulphanil-*p*-aminoanilide resulted, a compound which was of the same order of toxicity but twice as active by mouth against streptococci in mice.

Two other water-soluble derivatives of sulphanilamide, *p*-aminobenzenesulphonylethanolamine, and *p*-aminobenzenesulphonyl glycine, were possessed of little or no activity.

A number of derivatives of disulphanilamide were also prepared, but were all of decreased therapeutic efficiency, such as the monosodium salt, the water soluble ethanolamine and the glycine derivative. The trisulphanilamide and its sodium salt were also possessed of low therapeutic activity. Mayer (1937) reported that *p*-nitrobenzenesulphonamide was active against streptococci, though to a somewhat less degree than sulphanilamide. Among other derivatives of sulphanilamide is the highly soluble sodium salt of *p*-sulphamidophenylpyridine-β-carboxyamide-α-carboxylic acid, which was found to be of low toxicity by Hykes, Hykes and Rerabek (1937). A mouse of 20 gm. weight tolerates 100 mgm.



Trisulphanilamide

per os, while the rabbit can be given 3 gm. per kilogram of body weight. A single dose of 10 mgm. given to mice gave 10 per cent. of survivors when the mice were infected with hæmolytic streptococci, while a dose of 5 mgm. immediately after inoculation and repeated at the end of forty-eight hours produced cure in 30 per cent. of mice.

While *d*-glutamine and *p*-aminoglutamine have no protective action in streptococcal infections in rabbits, phenylalanine sulphonamide has been shown by Schaffer (1938) to be superior to sulphonamide: *p*-tolylsulphonamide on the other hand was inactive: 2 c.c. of a 1 per cent. solution of phenylalanine sulphonamide given four times a day for six days proved entirely non-toxic to rabbits and cured twelve out of twelve rabbits infected with hæmolytic streptococci. Mention must also be made of sodium sulphanilyl sulphanilate (*cf.* Dochez and Slanetz, 1938).

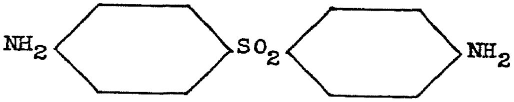
In an attempt to obtain water-soluble derivatives of *p*-aminobenzenesulphonamide a series of sulphonic acid compounds of *p*-aminobenzenesulphonamide were prepared by Smyth and Carpenter (1938). The compounds having a solubility in water greater than 1 in 20 were as follows:—

Compound.	Solubility in water at 25° C.	Per cent. of sulphanilamide in molecule.	Toxicity L.D.$_{50}$ for rats in mgm. per kilogram of body weight.
Sulphanilamide .	1 in 200	100	7·04
Camphorsulphonate .	1 ,, 2	42·5	6·24
Benzenesulphonate .	1 ,, 14	49·4	5·40
Phenolsulphonate .	1 ,, 14	49·7	7·04
Sulphosalicylate .	1 ,, 16	44·1	6·00

The protective action of these compounds against beta hæmolytic streptococci, Type A, was equal to or greater than that of sulphanilamide, although the derivatives contain only 40 to 50 per cent. of sulphanilamide. The protective action of the camphorsulphonate, most soluble of the compounds, was not due to the camphorsulphonic acid radical alone, since this failed to protect rats when administered in equivalent doses. Buttle *et al.* (1938) also studied some further derivatives of sulphanilamide. Thus 2-carboxybenzylideneaminobenzene-4-sulphonamide was found to be equally efficient in streptococcal infections in mice and less toxic, since doses of 100 mgm. were well tolerated by mouth. 4-Sulphonamidophenylguanidine is as active but also as toxic as sulphanilamide, while 4 : 4′-diacetylaminobenzenesulphonamide is inactive.

Diphenyl Sulphones and Sulphides

The compounds previously described can all be regarded as variations on a single theme, alterations in the molecule of *p*-aminobenzenesulphonamide. Two preparations of a different chemical structure were studied by Buttle, Stephenson, Smith, Dewing and Foster (1937), 4 : 4′-diaminodiphenylsulphone and 4 : 4′-dinitrodiphenylsulphone.



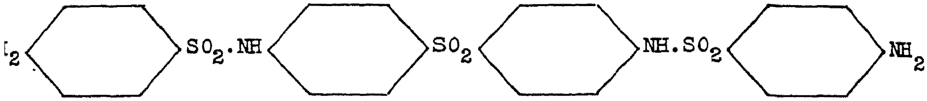
4 : 4′-Diaminodiphenylsulphone (diaminosulphone) was originally prepared by Fromm and Wittmann (1908), has a melting point of 176° C. and is soluble in water at room temperature only to the extent of 0·01 per cent., but in hot water slightly more soluble (0·05 per cent.). It occurs in very long colourless rectangular plates. 4 : 4′-Dinitrodiphenylsulphone is an amorphous yellowish powder, insoluble in water with a melting point of 238° C. (Fourneau *et al.*, 1937 [1]). 4 : 4′-Dinitrodiphenylsulphone is tolerated in doses of 200 mgm. by mouth, but 400 mgm. is lethal : doses of 40 mgm. can be given repeatedly to infected mice. 4 : 4′-Diaminodiphenylsulphone is tolerated in 5 mgm. doses by mouth : 2 mgm. can be given daily to normal or infected mice, but daily doses of 5 mgm. kill some of the animals. Infected mice are slightly more susceptible. Although the diaminodiphenylsulphone is about ten times as toxic as sulphanilamide when a single dose is given to normal mice, it is not more toxic to normal

rabbits and monkeys. Single doses of 2 gm. per kilogram of either of the drugs cause slight symptoms in the rabbit but not in the monkey. A single dose of 0·3 gm. of the diamino compound was taken by a healthy individual and caused no symptoms, but five hours afterwards the blood contained methæmoglobin. Sulphone was present in the urine in from twelve to eighteen hours after injection. Blood counts were normal. In mice toxic doses produce excitement and aimless running about: paralysis eventually follows, but the symptoms may persist for two or more days. Dilatation of the stomach and intestine is found post-mortem. In experiments with hæmolytic streptococcal infections in mice the diaminophenylsulphone in doses of 2 mgm. gave better results than 50 mgm. of sulphanilamide. In fact in mice the diaminodiphenylsulphone is one hundred times more effective than sulphanilamide. In rabbits infected with streptococci it is, however, only ten times as effective as sulphanilamide. This may be correlated with the fact that sulphanilamide is excreted twice as rapidly in the mouse as in the rabbit. This slower rate of excretion is also found in the monkey and in man. In pneumococcal infections of mice the diaminodiphenylsulphone in 2 mgm. doses is more active than sulphanilamide in 40 mgm. doses : it also appears to be effective in staphylococcal infections. The activity of the diaminodiphenylsulphone was also investigated at the same time by Fourneau *et al.* (1937 [1]) and Feinstone *et al.* (1938). Fourneau and his co-workers (1937 [1]) also found that *p*-(dinitrodiphenyl)-sulphone was somewhat less active against streptococci. Another compound originally prepared by Fromm and Wittmann (1908) was 4 : 4′-diacetylaminodiphenylsulphone, "Rodilone." The activity of this compound has been studied by Fourneau *et al.* (1937 [2]), Bauer and Rosenthal (1938), Cooper, Gross and Lewis (1938), Buttle *et al.* (1938) and Feinstone *et al.* (1938). Rodilone (1399F) appears to have a very low toxicity for mice, since 800 mgm. can be given to a 20 gm. mouse by mouth without causing any symptoms. When injected subcutaneously it causes local œdema with leucocytic infiltration in the neighbourhood of crystalline sulphone deposits. Its activity against streptococcal infections, while about ten times that of sulphanilamide, is equal to that of di-(*p*-nitrophenyl)-sulphone but less than that of di-(*p*-aminophenyl)-sulphone. In pneumococcal infections Fourneau *et al.* (1937 [2]) found it to be ten times as active as sulphanilamide, but this is denied by Buttle *et al.* (1938) (*cf* p. 385). In France this compound has been used in the treatment of gonorrhœa.

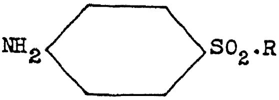
Buttle and his colleagues (1938) made the interesting observation that, despite the large doses tolerated by mice, no increase in therapeutic activity is obtainable with doses above 10 mgm. per day. This appears to be due to the slow rate of absorption or conversion into the active substance, possibly 4 : 4′-diaminodiphenylsulphone ; any excess is probably excreted unchanged. If treatment is delayed the therapeutic results in streptococcal infections in mice are not nearly so striking. When given by mouth to monkeys only minute traces of a diazotisable substance are found in the blood and the blood is not bactericidal. The urine, however, does contain such a diazotisable substance and will protect mice when given by mouth against experimental streptococcal infections. It would thus seem that the active substance is excreted as quickly as it is formed. It is, however, doubtful, therefore, whether this compound will be of value in acute infections, but in chronic conditions, owing to its low toxicity, it may possibly be of therapeutic value.

4 : 4′-Diacetyldihydroxydiphenylsulphone was found by Levaditi, Girard and Vaisman (1938) to be of little value against streptococcal infections in mice, although experimental meningococcal and, more especially, gonococcal infections (*cf.* p. 403) are cured. In addition, Bauer and Rosenthal (1938) reported that the formaldehyde sulfoxylate derivative of diaminodiphenylsulphone, which can be injected subcutaneously, is inferior to di-(*p*-acetylaminophenyl)sulphone, having a chemotherapeutic index in streptococcal infections of mice of 1 : 15 as compared with 1 : >20 ; for the diacetylsulphone the corresponding formaldehyde bisulphite derivative of diaminodiphenylsulphone is inactive.

Fourneau *et al.* (1938) have recently studied the effects of modifying the structure of 4 : 4′-diaminodiphenylsulphone. In particular, substitution in the amino group with the formyl COH, acetyl $COCH_3$, and propionyl radicals gives active compounds, as does a very different substitution in which the nuclei of diaminosulphone and *p*-aminobenzenesulphonamide are reunited. This compound (1468F) is thus related to uleron.

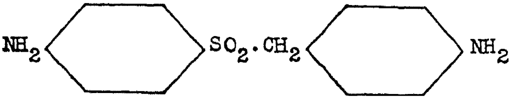


1468F is of low toxicity for the mouse and highly active against streptococcal infections. Acetylation, while decreasing toxicity, also decreases therapeutic activity. Replacing one of the two aromatic nuclei of 4 : 4′-diaminodiphenylsulphone by a fatty acid chain, it is found that in derivatives of the type :—

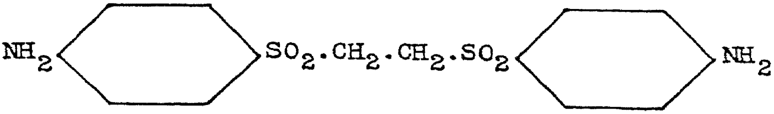


therapeutic action increases from the methyl chain $R = CH_3$, through $R = CH_2.CH_3$ to the propyl $R = CH_2.CH_2.CH_3$, and then decreases rapidly to the amyl chain where $R' = CH_2.CH_2.CH_2.CH_2.CH_3$. The methyl derivative is toxic *per os* for mice in doses of 50 mgm. per 20 gm. mouse and is curative in doses of 10 mgm. *p*-Aminobenzenepropylsulphone is toxic in doses of 20 mgm. per 20 gm. mouse and is only slightly less active than sulphanilamide itself. Compared with *p*-aminophenylpropylsulphone, *p*-nitrophenylpropylsulphide and *p*-aminophenylpropylsulphide are very inactive therapeutically. The *iso*propyl isomer and the corresponding allylic ($R' = CH_2.CH = CH_2$) and *iso*-butylic ($R = C_4H_9$) derivatives are all less active than the derivative with a normal chain.

The activity of *p*-aminobenzenemethylsulphone is not increased by substitution of a hydrogen of the terminal CH_3 group by the *p*-aminobenzene radical. Thus



is less active and



is quite inactive.

Having replaced one of the aromatic nuclei of 4 : 4′-diaminodiphenylsulphone by fatty chains without greatly reducing therapeutic activity, the dipropylsulphone and the corresponding sulphide were found to be quite inactive.

Modifications in the structure of 4 : 4′-diaminodiphenylsulphone have also been produced by Buttle, Gray and their collaborators (1938) with the object of reducing toxicity without at the same time decreasing the therapeutic activity in streptococcal infections in mice. Their results may be summarised as follows.

The following modifications were investigated :—

(1) Modification of the Sulphone Group

(*a*) **Diamino Derivatives.** 4 : 4′-Diaminodiphenylsulphoxide, investigated by Fourneau *et al.* (1937 [3]) and Levaditi and Vaisman (1937), is less active than the corresponding sulphone, and the sulphide still less active, while the toxicity is about the same. Acetylation of the amino-group of the sulphoxide does not reduce activity appreciably as it does in the case of the sulphone. The following compounds are either inactive or show decreased activity as compared with the sulphone : 4 : 4′-diacetylaminodiphenyldisulphone : 4 : 4′-diaminodiphenyldisulphoxide ; 4-aminophenyl-4′-aminobenzenesulphonate and the acetyl derivative, 4 : 4′-diaminodiphenyldisulphide ; 4 : 4′-diaminodiphenyloxide and 4 : 4′-diaminodiphenyldimethane.

(*b*) **Dinitro Derivatives.** Whilst 4 : 4′-dinitrodiphenylsulphone has about one-twentieth the activity and toxicity of the diamino compound, the sulphoxide has the same activity and toxicity as the corresponding sulphone and the sulphide is only very slightly active : 4 : 4′-dinitrodiphenylmethane, 4 : 4′-dinitrodiphenyloxide and 4 : 4′-dinitrodiphenyldisulphide are less active than diaminodiphenylsulphone.

(2) Substitution in the Amino Group

(*a*) **Acyl-Substituents.** 4 : 4′-Diacetylaminodiphenylsulphone has already been discussed on p. 372.

The dimyristyl and dilauryl derivatives of diaminodiphenylsulphone are inactive. The di-(aminobenzenesulphonyl)-derivative has one-hundredth of the activity of the parent substance. 4 : 4′-Disulphonamidodiphenylsulphone is quite inert.

(*b*) **Schiff's Bases.** Of a number of anils in which one or both of the amino groups of the diaminodiphenylsulphone are condensed with aromatic aldehydes the benzylidene compound is the most interesting. It is toxic in doses of 200 mgm. *per os* for a 20 gm. mouse, but a single dose of 50 mgm. is tolerated and repeated daily doses of 10 mgm. can be given to infected mice. It is half as active as the parent substance in streptococcal infections in mice and produces a bactericidal action on the blood of monkeys when given by mouth. It produces a better therapeutic effect in pneumococcal infections, Type I (Buttle, 1937) and Type II, of mice, than sulphanilamide or any other compound, and better in Type III than sulphanilamide, and is more active in 10 mgm. doses than 1 mgm. of the sulphone.

Anisylidene, piperonylidene, dicinnamylidene and dimethylaminobenzylidene derivatives have all about the same activity in streptococcal infections in mice as the benzylidene derivatives and are of the same or less toxicity. They do not induce a bactericidal effect in the blood of the monkey. Di-*o*-carboxybenzylideneaminodiphenylsulphone has only about one-hundredth the activity of the parent substance.

The sodium bisulphite compound of 4 : 4′-dicinnamylideneaminodiphenylsulphone is freely soluble in water and has about one-twentieth of the therapeutic activity of the parent substance when given by mouth ; when given subcutaneously, though slightly more toxic, it is also slightly more effective than an equal quantity of the corresponding derivative of sulphanilamide. A very soluble substance is obtained by heating 4 : 4′-diaminodiphenylsulphone with an aqueous solution of glucose. By mouth 50 mgm. is tolerated and 100 mgm. is lethal for 20 gm. mice : subcutaneously it is less active and also less toxic, daily doses of 150 mgm. being well tolerated. Like the benzylidene Schiff's base, it produces a good therapeutic effect in pneumococcal infections in mice. (*cf.* p. 385).

(*c*) **Aralkyl Derivatives.** Tetrabenzyldiaminodiphenylsulphone is inactive.

(3) Additional Substituents

Additional substituents, *e.g.*, nitro-, chloro- and amino-groups, all have a dystherapeutic effect.

(4) Substituents other than Amino Groups.

4 : 4′-Dichlordiphenylsulphone, 4 : 4′-dihydroxydiphenylsulphone and 4 : 4′-dihydroxy-3-3′-dinitrodiphenylsulphone are completely inactive.

(5) Unsymmetrical Compounds.

4-Nitro-4-aminodiphenylsulphone (*cf.* Fourneau *et al.*, 1937 [3]) has rather more than half the activity of the diamino compound and the same toxicity, 4-aminobenzylphenylsulphone is inactive, 4-aminophenylbenzylsulphone very slightly active. In each case the nitro- and amino-derivatives have equal activity and the presence of a further amino- or nitro-group in the *para*-position in the above amino- or nitro-compounds does not influence activity except in the case of 4-nitrobenzyl-4′-nitrophenylsulphone, which is inactive.

The series of compounds of the general formula —(NH_2) or —$(NO_2).C_6H_4.SO_2R$ prepared by Waldron and Reid (1923), in which R is an aliphatic chain of 1 to 4 carbon atoms, shows that when the amino-compounds contain 2 or 3 carbon atoms in the chain there is a trace of activity, whereas those with 1 or 4 carbon atoms are inactive (Fourneau *et al.*, 1938, and Buttle *et al.*, 1938). Ethyl- and propyl-sulphones have the same activity as 4-aminodiphenylsulphone. On the other hand, 4-nitrodiphenylsulphone is as active as the corresponding amino-compound, whereas the aliphatic nitro-compounds are inactive. In comparison with 4 : 4′-diaminodiphenylsulphone the following compounds were quite inactive : 2 : 5-dihydroxy-4′-acetylaminodiphenylsulphone ; 4-nitrophenylmercaptan, tetramethyldiaminobenzophenone ; 4 : 4′-diaminodiphenylcarbamide ; 4-aminobenzenesulphonylglycine (*cf.* Bauer and Rosenthal, 1938), while the following compounds were slightly active (the relative activity is shown in brackets): 2 : 5-dihydroxy-4′-aminodiphenylsulphone (< 1/1,000), benzyl-4-acetylaminophenylsulphone (1/1,000), 2 : 2′-dinitro-4 : 4′-diaminodiphenylmethane (1/5,000).

The pharmacology of the aminophenylsulphones has been studied by Nitti, Bovet and Hamon (1938), who have found that when given by mouth to rabbits 4 : 4′-diaminodiphenylsulphone is rapidly absorbed while the blood concentration reaches a maximum eight to ten hours afterwards. At the end of forty-eight hours the drug is no longer detectable in the blood stream. Feinstone *et al.* (1938) noted that mice receiving by mouth 4 or more milligrams of the compound per mouse had essentially the same blood levels at the end of one hour. Excretion takes place in the urine partly in the form of the acetyl derivative. It appears that the higher members of the series in which NH_2 is replaced by either $NH.COH$ $NHCOCH_3$, $NHCOCH_2.CH_3$ or $NH.COCH_2.CH_2.CH_3$, are all rapidly hydrolysed in the tissues to the amino-group. The excretion of diaminosulphones in the urine is greatest when the number of carbon atoms in the hydrocarbon chain attached to the amine is unequal.

Thus the urinary concentration is :—

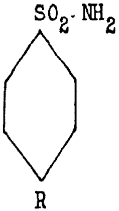
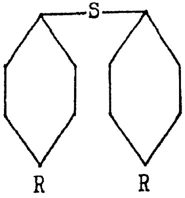
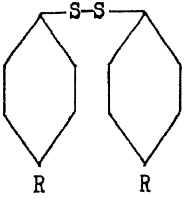
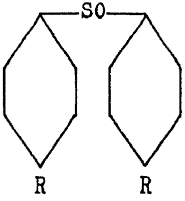
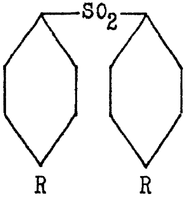
360	mgm.	per 100	c.c.	with	$NH.COH$.
100	,,	,,	,,	,,	$NH.COCH_2.CH_3$.
5–10	,,	,,	,,	,,	$NH.COCH_3$.
50	,,	,,	,,	,,	$NH.COCH_2.CH_2.CH_3$.

In the series of diaminosulphones, as in the sulphonamide series, activity is due to the derivative with a free amino-group.

Antistreptococcic action has also been obtained with 4 : 4′-dinitrodiphenylsulphide and 4 : 4′-dinitrodiphenyldisulphide, derivatives of 4 : 4′-diaminodiphenylsulphide, the activity of which has been investigated by Fourneau and his co-workers (1937 [1]). Gley (1937) claimed that *p*-acetylaminophenylsulphone and *p*-acetylaminothiophenol are almost as active as rubiazol, but this observation has not been confirmed.

Levaditi and Vaisman (1937) observed activity in *p*-diamino-

MAXIMUM TOLERATED DOSES AND CURATIVE DOSES FOR STREPTOCOCCAL (S) AND PNEUMOCOCCAL (P) INFECTIONS IN MICE (FOURNEAU *et al.* 1937 [3]).

	Benzene-sulphonamides.	Diphenylsulphides.	Diphenyldisulphides.	Diphenylsulphoxides.	Diphenylsulphones.
					
$R = NH_2$					
Max. tol. dose =	0·050	0·010	0·001	0·010	0·002
S =	0·001	0·001	inactive	0·001	0·0005
P =	0·010	0·01	„	0·001	0·001
$R = NHCOCH_3$					
Max. tol. dose	>0·200	>0·200	0·100	0·050	>0·200
S =	0·010	0·001	0·010	0·001	0·00005
P =	0·010	0·001	0·010	0·001	0·001
$R = NO_2$					
Max. tol. dose =	0·005	>0·200	0·100	0·050	0·050
S =	0·005	0·001	0·005	0·001	0·0001
P =	0·005	0·001	0·005	0·001	0·001

diphenylsulphoxide and its derivatives, more especially nitroaminodiphenylsulphoxide (*cf.* Girard, Ray and Richard, 1937), while Buttle *et al.* (1938) found that of the two optically active forms into which 4-aminodiphenylsulphoxide can be resolved it is the *l*-isomer which is far the more active.

Of the active compounds so far described, benzenesulphonamides, diphenylsulphides, diphenyldisulphides, diphenylsulphoxides and diphenylsulphones, each exists in the form of the *p*-amino, *p*-acetylamino and *p*-nitro derivatives. The relationships of the *p*-amino, *p*-acetamino and *p*-nitro derivatives are shown in the table (Fourneau *et al.*, 1937 [3]), together with the maximum tolerated dose for mice in gm. *per os* per 20 gm. of body weight and the approximate curative doses for hæmolytic streptococci (S) and pneumococci (P) are recorded.

References

BARLOW, O. W., 1937, Relative toxicities and therapeutic values of three chemotherapeutic agents of the sulphonamide type. *Proc. Soc. exp. Biol., N.Y.*, **XXXVII.**, 315.

BAUER, H. S., and ROSENTHAL, S. M., 1938, Studies in chemotherapy. VII. Some new sulphur compounds active against bacterial infections. *Publ. Hlth. Rep., Wash.*, **LIII.**, 40.

BUTTLE, G. A. H., 1937, Discussion on the treatment of bacterial diseases with substances related to sulphanilamide. *Proc. R. Soc. Med.*, **XXXI.**, 154.

BUTTLE, G. A. H., DEWING, T., FOSTER, G. E., GRAY, W. H., SMITH, S., and STEPHENSON, D., 1938, The action of substances allied to 4 : 4′-diaminodiphenylsulphone in streptococcal and other infections in mice. *Biochem. J.*, **XXXII.**, 1101.

BUTTLE, G. A. H., GRAY, W. H., and STEPHENSON, D., 1936, Protection of mice against streptococcal and other infections by *p*-aminobenzenesulphonamide and related substances. *Lancet*, *i.*, 1286.

BUTTLE, G. A. H., STEPHENSON, D., SMITH, S., DEWING, T., and FOSTER, G. E., 1937, The treatment of streptococcal infections in mice with 4 : 4′-diaminodiphenylsulphone. *Ibid.*, *i.*, 1331.

COOPER, F. B., GROSS, P., and LEWIS, M., 1938, Sulfone and sulfonanilide therapy in streptococcal infections. *Proc. Soc. exp. Biol., N.Y.*, **XXXVIII.**, 375.

DOCHEZ, A. R., and SLANETZ, C. A., 1938, The treatment of canine distemper with a chemotherapeutic agent, sodium sulfanilyl sulfanilate. *Science*, **LXXXVII.**, 142.

DOMAGK, G., 1935, Chemotherapie der bakteriellen Infektionen. *Z. angew. Chem.*, **XLVIII.**, 657.

DOMAGK, G., 1936, Chemotherapie der Streptokokkeninfektionen. *Klin. Wschr.*, **XV.**, 1585.

Domagk, G., 1937, Weitere Untersuchungen über die chemotherapeutische Wirkung sulfonamidhaltiger Verbindungen bei bakteriellen Infektionen. *Klin. Wschr.*, **XVI.**, 1412.

Feinstone, W. H., Bliss, E. A., Ott, E., and Long, P. H., 1938, Observations concerning the toxicity, absorption and therapeutic effect of sulphanilamide and certain related organic sulphur-containing compounds in experimental infections in mice. *Bull. Johns Hopk. Hosp.*, **LXII.**, 565.

Fourneau, E., Tréfouël, J., Tréfouël, Mme., Nitti, F., and Bovet, D., 1936 Chimiothérapie des infections streptococciques par les dérivés du *p*-aminophenyl sulfamide. *C. R. Soc. Biol., Paris*, **CXXII.**, 258.

Fourneau, E., Tréfouël, J., Tréfouël, Mme., Nitti, F., and Bovet, D., 1937^1, Action antistreptococcique des dérivés sulfurés organiques. *C. R. Acad. Sci. Paris*, **CCIV.**, 1763.

Fourneau, E., Tréfouël, J., Tréfouël, Mme., Nitti, F., and Bovet, D., 1937^2, Chimiothérapie de l'infection pneumococcique par la di-(*p*-acétylaminophényl)-sulfone (1399F). *Ibid.*, **CCV.**, 299.

Fourneau, E., Tréfouël, J., Tréfouël, Mme., Nitti, F., and Bovet, D., 1937^3, Rapport entre la constitution chimique et l'activité thérapeutique antimicrobienne des dérivés organiques du soufre : phénylsulfamides, diphénylsulfures, diphénylsulfones. *Bull. Acad. Méd., Paris*, **CXVIII.**, 210.

Fourneau, E., Tréfouël, J., Tréfouël, Mme., Nitti, F., and Bovet, D., 1938, Action antistreptococcique des *p*-aminophénylalcoylsulfones et de quelques dérivés sulfurés voisins. *C. R. Soc. Biol., Paris*, **CXXVII.**, 393.

Fromm, E., and Wittmann, J., 1908, Derivate des *p*-Nitrothiophenols. *Ber. dtsch. chem. Ges.*, **XLI.**, 2264.

Girard, A., Ray, A., and Richard, G., 1937, Antimicrobial action of some aromatic compounds. *Nature*, **CXL.**, 283.

Gley, P., 1937, Action antistreptococcique de certains dérivés sulfurés organiques. *C. R. Acad. Sci., Paris*, **CCIV.**, 1907.

Gley, P., and Girard, A., 1937, Action antistreptococcique de la thioaniline. *C. R. Soc. Biol., Paris*, **CXXV.**, 1027.

Goissedet, P., Despois, R., Gaillot, P., and Mayer, R. L., 1936, De l'action du radical sulfamide SO_2NH_2 sur l'infection streptococcique expérimentale de la souris. *Ibid.*, **CXXI.**, 1082.

Gray, W. H., Buttle, G. A. H., and Stephenson, D., 1937, Derivatives of *p*-aminobenzenesulphonamide in the treatment of streptococcal infection in mice. *Biochem. J.*, **XXXI.**, 724.

Halpern, B. N., and Mayer, R. L., 1936, Toxicité expérimentale comparée de quelques substances antistreptococciques. *Pr. méd.*, **XLV.**, 747.

Heidelberger, M., and Jacobs, W. A., 1919, Syntheses in the cinchona series. III. Azo dyes derived from hydrocupreine and hydrocupreidine. *J. Amer. chem. Soc.*, **XLI.**, 2131.

Hykes, O. V., Hykes, D. E., and Rerabek, J., 1937, Action spécifique d'un nouveau dérivé de la *p*-aminophénylsulfamide sur la septicémie streptococcique expérimentale. *C. R. Soc. Biol., Paris*, **CXXVI.**, 635.

Levaditi, C., Girard, A., and Vaisman, A., 1938, Chimiothérapie des infections expérimentales (gonocoque, méningocoque, pneumocoque et streptocoque) par la diacetyldioxydiphénylsulfone. *Ibid.*, **CXXVIII.**, 19.

Levaditi, C., and Vaisman, A., 1935, Action curative et préventive du chlorhydrate de 4′-sulfamide-2 : 4-diaminoazobenzène et de quelques dérivés similaires dans la streptococcie expérimentale. *Pr. méd.*, **XLIII.**, 2097.

Levaditi, C., and Vaisman, A., 1937, La toxi-infection gonococcique expérimentale et son traitement chimiothérapique. *Ibid.*, **XLV.**, 1371.

Mayer, R. L., 1937, Recherches sur le mécanisme de l'action antistreptococcique de l'aminobenzènesulfamide et de ses dérivés. *Bull. Acad. Méd., Paris*, **CXVII.**, 727.

Nitti, F., Bovet, D., and Hamon, V., 1938, Le sort des aminophénylsulfones dans l'organisme et leur activité antimicrobienne. *C. R. Soc. Biol., Paris*, **CXXVIII.**, 26.

Raiziss, G. W., Severac, M., Moetsch, J. C., and Clemence, L. W., 1938, The chemotherapy of sulfanilamide, prontosil soluble, di-sulfanilamide and proseptasine. *J. Chemotherap.*, **XIV.**, 91.

Rosenthal, S. M., Bauer, H., and Branham, S. E., 1937, Studies in chemotherapy. IV. Comparative studies of sulfonamide compounds in experimental pneumococcus, streptococcus and meningococcus infections. *Publ. Hlth. Rep., Wash.*, **LII.**, 662.

Schaffer, C. W., 1938, Protective effects against lethal *Streptococcus hemolyticus* of glutamine and various sulfonamide compounds. *Proc. Soc. exp. Biol., N.Y.*, **XXXVII.**, 648.

Smyth, H. F., and Carpenter, C. P., 1938, Water-soluble derivatives of *p*-aminobenzenesulfonamide (sulfanilamide). *Science*, **LXXXVII.**, 350.

Waldron, W. R., and Reid, E. E., 1923, The influence of sulfur on the colour of azo dyes. *J. Amer. chem. Soc.*, **XLV.**, 2399.

Whitby, L. E. H., 1937, An experimental assessment of the therapeutic efficacy of amine compounds with special reference to *p*-benzylaminobenzenesulphonamide. *Lancet*, *i.*, 1517.

PNEUMOCOCCAL INFECTIONS

(*a*) Experimental Investigations

The first observations on pneumococcal infections in mice were made by Domagk (1935), who found that prontosil had a slight action on pneumococcus Type III but none on Types I and II. Later (1936) he reported that sulphanilamide was more active than prontosil against pneumococci.

Experimentally Buttle, Gray and Stephenson (1936) found that pneumococcal infections (Type I) of mice were hardly affected by sulphanilamide, while later observations (Buttle, Parish, McLeod and Stephenson, 1937, and Buttle, 1937) emphasised that sulphanilamide merely delays death in mice infected with pneumococci (Types I and II). Rosenthal (1937) reported that, in mice, sulphanilamide was active against seven strains of pneumococcus of Types I, II and III, although there was considerable variation in susceptibility to different strains. Cooper, Gross and Mellon (1937) found that sulphanilamide was to some extent effective against ten lethal doses of a highly virulent Type III pneumococcus inoculated subcutaneously in mice. Cooper and Gross (1937 [1]) also observed some degree of protection against the intratracheal inoculation of Type III pneumococcus in rats, as well as against two strains of Type II (Cooper and Gross, 1937 [2]) and of Type I (Gross and Cooper, 1937). Schmidt (1937) reported that sulphanilamide was effective against ten lethal doses of Type XIV pneumococci, against which no antisera are available, but Whitby (1937) observed practically no protection with 10,000 lethal doses of Type I pneumococcus injected intraperitoneally. Cooper *et al.* (1938 [3]) reported protection against Type VII pneumococci. Kreidler (1937) found that sulphanilamide had some curative action in pneumococcal Type I infections in rabbits, but the 100 per cent. protection found by Rosenthal, Bauer and Branham (1937) to be exerted in Type II and III infections in rats has not been fully confirmed.

Cooper, Gross and Lewis (1938[1]) produced a cerebrospinal meningitis in rats with a Type II pneumococcus. Oral treatment with sulphanilamide, instituted after the disease was well established, resulted in 73·3 per cent. of cures as compared with 100 per cent. mortality in untreated controls.

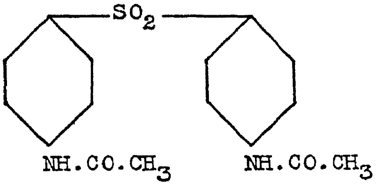
In vitro Nitti, Bovet and Depierre (1937) found that sulphanilamide had an inhibitory effect on pneumococcus, while Britton (1938) noted a distinct but variable bactericidal effect on Type I, Type II and Group IV pneumococci. On Type I pneumococcus, sulphanilamide was bacteriostatic in a dilution of 1 : 4,000, soluseptasine in a dilution of 1 : 160, proseptasine 1 : 50,000, 4 : 4′-diaminobenzenesulphonamide 1 : 17,600 and 4 : 3′-diaminobenzenesulphonamide 1 : 20,000. Rosenthal (1937) notes that no parallelism exists between the action *in vivo* and the bacteriostatic action *in vitro*.

It will be seen that the action of sulphanilamide on pneumococci either *in vitro* or *in vivo* is not nearly as striking as on streptococci. Branham and Rosenthal (1937) claim that sulphanilamide and immune serum together are superior to drug or serum therapy alone in the treatment of Type I pneumococcal infections in mice. Cooper and Gross (1937[2]), however, observed no advantage in combining immune serum and sulphanilamide in Type II infections in rats. Osgood and Brownlee (1938) found that against pneumococci Type I added to *in vitro* cultures of human bone marrow, sulphanilamide and immune serum combined were superior to either alone.

A number of other substances have also been investigated for pneumococcicidal action. Thus Domagk (1935) showed that the sodium salt of 4′-sulphonamidophenylazo-1-hydroxy-7-acetylaminonaphthalene-3 : 6-disulphonic acid was active against certain strains of pneumococcus. Buttle (1937) and Buttle *et al.* (1938) found that the benzylidene Schiff's base of diaminodiphenylsulphone was more effective than sulphanilamide in prolonging the life of mice infected with Types I and II pneumococci ; the compound, however, was four times as toxic as sulphanilamide. Whitby (1937) reported that 4 : 4′-diaminobenzenesulphonanilide tartrate and 4 : 3′-diaminobenzenesulphonanilide were approximately three times as efficient as sulphanilamide in protecting mice against 10,000 lethal doses of Type I pneumococci. Bauer and Rosenthal (1938), however, found 4 : 4′-diaminobenzenesulphonanilide inferior to sulphanilamide. Cooper *et al.* (1938[3]) at times obtained better results with this compound than with sulphanilamide, but regarded it as too toxic for clinical use (*cf.* Cooper *et al.*, 1938 [2]).

Uleron is said by Osterholz (1938) to be active in curing pneumococcal infections in mice, more especially those due to Type III organisms.

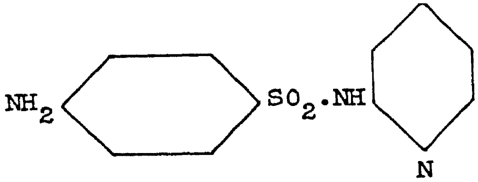
Buttle *et al.* (1937), Fourneau *et al.* (1937 [2]), and Feinstone *et al.* (1938) have all found that 4 : 4′-diaminodiphenylsulphone has a real degree of therapeutic efficiency in experimental infections of mice with Types I and II pneumococci. The toxicity is high, for from 0·35 to 0·45 gm. per kilogram of body weight causes a 50 per cent. mortality in mice. This toxicity probably precludes its use in human beings. In experimental meningitis in rats due to Type II pneumococci the percentage of cures was only 40 per cent. as compared with 73·3 per cent. with sulphanilamide (Cooper, Gross and Lewis, 1938 [1]). Fourneau *et al.* (1937 [2]) found that 4 : 4′-diacetylaminodiphenylsulphone (1399F) was ten times more active against pneumococcal infections in mice than sulphanilamide : 0·5 gm. per kilogram protected mice against pneumococci. Bauer and Rosenthal (1938) obtained similar results, but noted differences with different strains of pneumococci.



4 : 4′-*diacetylaminodiphenylsulphone*

Buttle *et al.* (1938), however, observed that this compound caused only a slight delay in the time of death of mice infected with pneumococci. Cooper *et al.* (1938 [3]) confirmed these results, since this compound was less effective than sulphanilamide against pneumococcal infections in mice caused by a Type VII and a Type III strain, as well as in pneumococcal pneumonia in rats caused by the same Type III strain. Levaditi *et al.* (1938) found 4 : 4′-diacetyldihydroxydiphenylsulphone to be only slightly active in pneumococcal infections of mice.

Whitby (1938) has recently investigated the chemotherapeutic action of 2(*p*-aminobenzenesulphonamido) pyridine in pneumococcal infections in mice. This compound (T. or M. and B. 693) is a white crystalline, almost tasteless solid, soluble in water at ordinary temperatures to the extent of approximately 1 in 1,000.



2(p-*aminobenzenesulphonamido*) *pyridine*

It differs from sulphanilamide in that one hydrogen of the SO_2NH_2 group is replaced by a basic pyridine group. The substance is also related to *p*-aminobenzenesulphonanilide, which has been reported as active against streptococci. According to Wien (1938), the average lethal dose is 16·6 gm. per kilogram of body weight for mice and 15·8 gm. per kilogram of body weight for rats. Doses of 1 gm. administered daily, for seven days, to cats and dogs appear to have no effect on the blood, urine or general health : administered to rats in doses of 3 mgm. per gm. for fifteen days it causes no increase in the excretion of porphyrins in the urine. In experimental infections in mice M. and B. 693 was found to be effective in doses of from 0·25 to 2 gm. per kilogram of body weight, from six to eight times smaller than the average lethal dose. Infections due to pneumococci of Types I, VII and VIII were especially susceptible to the action of 2-(*p*-aminobenzenesulphonamido) pyridine, while considerable action was also exerted on Types II, III and V. A definite action appears to be exerted on the capsules of the pneumococcus, which become swollen, crenated and eventually disappear, while, as previously observed by Buttle (1937) with other compounds, mice which have recovered as a result of drug treatment are immune to even a million lethal doses. 2-(*p*-Aminobenzenesulphonamido) pyridine is also highly protective against meningococci and against hæmolytic streptococci even in doses of 1 to 2 gm. per kilogram of body weight. The only other compound found to have an action on pneumococcal infections in mice is a very soluble substance prepared by Buttle *et al.* (1938) by heating 4 : 4′-diaminodiphenylsulphone with an aqueous solution of glucose. By mouth 100 mgm. is lethal for a 20 gm. mouse, but 50 mgm. is well tolerated : subcutaneously it is less active and also less toxic. In its action on pneumococci it is only very slightly less active than the benzylidene Schiff's base.

(*b*) Treatment of Human Infections

Clinical observations on the treatment of pneumococcal infections in man with sulphanilamide or prontosil have not been numerous. Millett (1937 [1]) records a case of Type III pneumococcus pneumonia in which a crisis was induced by sulphanilamide twenty-four hours after the onset of the disease. The only extensive series of lobar pneumonia cases so far reported (Heintzelmann, Hadley and Mellon, 1937) were all due to pneumococcus Type III and were treated with sulphanilamide and occasional injections of prontosil soluble. Nineteen patients were divided into two groups, nine being treated and ten used as controls. In the treated group the two oldest patients, aged sixty and seventy, died, while in the untreated group only two, aged thirty-one and thirty-nine, recovered. The treated group was, however, slightly favoured since the average age was 44·4 years as against 49·2 years for the untreated group. The latter group also included one alcoholic, one case with asthma and one case of slight myocarditis. In another series of Type III pneumonia occurring in Pittsburg, but not treated with sulphanilamide, twenty-four patients died and only nine recovered. The mortality for the treated patients was 22 per cent. as against 74 per cent. for the untreated. The evidence, therefore, suggests that in cases of lobar pneumonia due to pneumococcus Type III sulphanilamide is of value.

Louis (1938) successfully treated 2 cases of lobar pneumonia due to Type III organisms, 2 cases due to Type II, 1 case due to Type I and 1 case due to Type VIII pneumococci.

Somewhat similar results have been reported in pneumococcal meningitis. A case of pneumococcal meningitis (Millett, 1937 [2]) was treated by the intrathecal injection of prontosil soluble. At the subsequent post-mortem no gross or microscopical injury in the brain could be attributed to the use of protonsil soluble. Basman and Perley (1937) reported the successful treatment with sulphanilamide of one case of pneumococcal meningitis due to Type V pneumococci, two others of Type III died. Latto (1938) obtained a cure after treatment with prontosil. Allan *et al.* (1938) reported the cure of three cases due to Types XIV, XX and XXIX respectively. Query (1938) successfully treated a Type VII pneumococcal meningitis with sulfanilamide and specific serum. Landon (1938) gave intramuscular injections of prontosil soluble, 10 c.c. twice daily for four days, and cured a girl aged five years. Of 33 cases of pneumococcal meningitis up to the present treated with sulphonamide compounds (*cf.* Caldwell and Byrne, 1937; Mertins and Mertins, 1937; Neal *et al.*, 1937; Latto, 1938; Allan *et al.*, 1938; Young, 1938; Finland *et al.*, 1938), 17 have recovered, a somewhat striking result, since before the introduction of these remedies practically no recoveries had ever been recorded. No cases of pneumococcal peritonitis treated with sulphanilamide have yet been reported.

The most remarkable successes have been obtained with M. and B. 693.

Telling and Oliver (1938) record a striking result in a patient infected with Type III pneumococci and suffering from massive collapse of the lungs. In three other patients in which Types III, XI and I pneumococci were concerned administration of the drug was followed by decapsulation of the pneumococci with consequent loss of type specificity and disappearance of the organisms from the sputum. The most extensive series of cases is, however, that recorded by Evans and Gaisford (1938), who treated 100 patients suffering from lobar pneumonia. Only eight patients died, whereas the death rate in 100 controls was 27 per cent. In 44 cases where it was possible to type the organisms the results were: Type I, 28; Type II, 8; Type III, 3; and Group IV, 5. In the case of the first 60 patients the treatment consisted of 0·5 gm. at four-hourly intervals for from three to four days, then 0·5 gm. twice daily for two or three days, giving an average total dose of 12 gm. The second 40 patients received 2 gm. on admission and thereafter 1 gm. four-hourly, making an average total dose of 25 gm. The maximum amount given in twenty-four hours was 9 gm. The fall in temperature and the rapid disappearance of toxic symptoms, especially delirium, were very noticeable. In 40 cases of bronchopneumonia there were only two deaths, both of patients with long-standing pneumococcal peritonitis. Toxic manifestations were infrequent, though cyanosis and methæmoglobinæmia, but never sulphæmoglobinæmia, were seen in about a quarter of the patients. No evidence of renal damage was forthcoming, while a child of three received 1 gm. followed by 0·5 gm. six-hourly without any ill effects. A patient with pneumococcal septicæmia was also cured by this compound (Dyke, 1938), while at least three cases of pneumococcal meningitis have also been successfully treated (*cf.* Reid and Dyke, 1938, and Robertson, 1938).

Lloyd, Erskine and Johnson (1938) report a number of toxic reactions, including anorexia, nausea, vomiting, diarrhœa, headache, depression and dizziness, skin eruptions and leucopenia. Sulphæmoglobinæmia and methæmoglobinæmia were not seen.

Fleming (1938) finds that *in vitro* 2-(*p*-aminobenzenesulphonamido) pyridine inhibits the growth of pneumococci and hæmolytic streptococci in human blood in concentrations which, it is reasonable to suppose, are obtained therapeutically. Leucocytes are necessary for this destruction, which is enhanced by the presence of immune bodies ; hence the advisability of combining the drug with active or passive immunisation.

References

Allan, W. B., Mayer, S., and Williams, R., 1938, Pneumococcus meningitis : a report of three cases. *Amer. J. med. Sci.*, **CXCVI.**, 99.

Basman, J., and Perley, A. M., 1937, Report of patients treated with sulfanilamide at St. Louis Children's Hospital. *J. Pediat.*, **XI.**, 212.

Bauer, H., and Rosenthal, S. M., 1938, Studies in chemotherapy, VII. Some new sulphur compounds active against bacterial infections. *Publ. Hlth. Rep., Wash.*, **LIII.**, 40.

Branham, S. E., and Rosenthal, S. M., 1937, Studies in chemotherapy. V. Sulphanilamide, serum and combined drug and serum therapy in experimental meningococcus and pneumococcus infections in mice. *Ibid.*, **LII.**, 685.

Britton, C. J. C., 1938, The effect of sulphonamide compounds on certain bacteria *in vitro*. *Brit. J. exp. Path.*, **XIX.**, 140.

Buttle, G. A. H., 1937, Discussion on the treatment of bacterial diseases with substances related to sulphanilamide. *Proc. R. Soc. Med.*, **XXXI.**, 154.

Buttle, G. A. H., Dewing, T., Foster, G. E., Gray, W. H., Smith, S., and Stephenson, D., 1938, The action of substances allied to 4 : 4′-diaminodiphenylsulphone in streptococcal and other infections in mice. *Biochem. J.*, **XXXII.**, 1101.

Buttle, G. A. H., Gray, W. H., and Stephenson, D., 1936, Protection of mice against streptococcal and other infections by *p*-aminobenzenesulphonamide and related substances. *Lancet*, *i.*, 1286.

Buttle, G. A. H., Parish, H. J., McLeod, M., and Stephenson, D., 1937, The chemotherapy of typhoid and some other non-streptococcal infections in mice. *Ibid.*, *i.*, 681.

Buttle, G. A. H., Stephenson, D., Smith, S., Dewing, T., and Foster, G. E., 1937, The treatment of streptococcal infections in mice with 4 : 4′-diaminodiphenylsulphone. *Ibid.*, *i.*, 1331.

Caldwell, J. R., and Byrne, P. S., 1937, Recovery from pneumococcal meningitis. *Brit. med. J.*, *i.*, 1204.

Cooper, F. B., and Gross, P., 1937^{1}, *para*-Aminobenzenesulfonamide therapy in experimental type III pneumococcal pneumonia. *Proc. Soc. exp. Biol., N.Y.*, **XXXVI.**, 678.

Cooper, F. B., and Gross, P., 1937^{2}, Sulfanilamide antipneumococcus serum and vitamin C therapy in Type II pneumococcal pneumonia of rats. *Ibid.*, **XXXVI.**, 774.

Cooper, F. B., Gross, P., and Lewis, M., 1938^{1}, Chemotherapy of pneumococcal (Type II) meningitis in the rat. *Ibid.*, **XXXVIII.**, 835.

Cooper, F. B., Gross, P., and Lewis, M., 1938^{2}, Sulfone and sulfonanilide therapy in streptococcal infections. *Ibid.*, **XXXVIII.**, 375.

Cooper, F. B., Gross, P., and Lewis, M., 1938^{3}, Chemotherapy of Types VII and III pneumococcal infections with sulphanilamide, 4,4′-di(acetylamino)-diphenylsulphone and 4, 4′-diaminobenzenesulphonamide. *Amer. J. med. Sci.*, **CXCVI.**, 343.

Cooper, F. B., Gross, P., and Mellon, R. R., 1937, Action of sulfanilamide on Type III pneumococcus infections in mice. *Proc. Soc. exp. Biol., N.Y.*, **XXXVI.**, 148.

Domagk, G., 1935, Ein Beitrag zur Chemotherapie der bakteriellen Infektionen. *Dtsch. med. Wschr.*, **LXI.**, 250.

Domagk, G., 1936, Chemotherapie der Streptokokkeninfektionen. *Klin. Wschr.*, **XV.**, 1585.

Dyke, S. C., 1938, Pneumococcal septicæmia treated with M. & B. 693. *Lancet*, *ii.*, 621.

Evans, G. M., and Gaisford, W. F., 1938, Treatment of pneumonia with 2-(*p*-aminobenzenesulphonamido) pyridine. *Ibid.*, *ii.*, 14.

Feinstone, W. H., Bliss, E. A., Ott, E., and Long, P. H., 1938, Observations concerning the toxicity, absorption and therapeutic effect of sulphanilamide and certain related organic sulphur-containing compounds in experimental infections in mice. *Bull. Johns Hopk. Hosp.*, **LXII.**, 565.

Finland, M., Brown, J. W., and Rauh, A. E., 1938, Treatment of pneumococcic meningitis : study of ten cases treated with sulfanilamide alone or in various combinations with specific antipneumococcus serum and complement, including six recoveries. *New Eng. J. Med.*, **CCXVIII.**, 1033.

FLEMING, A., 1938, The antibacterial action *in vitro* of 2-(*p*-aminobenzenesulphonamido) pyridine on pneumococci and streptococci. *Lancet, ii.*, 74.

FOURNEAU, E., TRÉFOUËL, J., TRÉFOUËL, Mme., NITTI, F., and BOVET, D., 1937[1], Chimiothérapie de l'infection pneumococcique par la di-(*p*-acetylaminophényl)-sulfone (1399F). *C. R. Acad. Sci., Paris*, CCV., 299.

FOURNEAU, E., TRÉFOUËL, J., TRÉFOUËL, Mme., NITTI, F., and BOVET, D., 1937[2], Rapport entre la constitution chimique et l'activité thérapeutiques antimicrobienne des dérivés organique du soufre : phenylsulfamides, diphenylsulfures, diphenylsulfones. *Bull. Acad. Med.*, CXVIII., 210.

GROSS, P., and COOPER, F. B., 1937, *p*-aminobenzenesulfonamide and antipneumococcal serum therapy in Type I pneumococcal infections of rats. *Proc. Soc. exp. Biol., N.Y.*, XXXVI., 535.

HEINTZELMANN, J. H. L., HADLEY, P., and MELLON, R. R., 1937, The use of *p*-aminobenzenesulphonamide in Type III pneumococcus pneumonia. *Amer. J. med. Sci.*, CXCIII., 759.

KREIDLER, W. A., 1937, Treatment of pneumococcal infections in rabbits with sulfanilamide. *Proc. Soc. exp. Biol., N.Y.*, XXXVII., 146.

LANDON, J., 1938, Pneumococcal meningitis treated with prontosil soluble. *Brit. med. J., i.*, 844.

LATTO, C., 1938, Pneumococcal meningitis treated with prontosil. *Ibid., i.*, 566.

LEVADITI, C., GIRARD, A., and VAISMAN, A., 1938, Chimiothérapie des infections expérimentales (gonocoque, méningocoque, pneumocoque et streptocoque). *C. R. Soc. Biol., Paris*, CXXVII., 19.

LLOYD, V. E., ERSKINE, D., and JOHNSON, A. G., 1938, Chemotherapy of pneumococcal and other infections. *Lancet, i.*, 1305.

LOUIS, D. J., 1938, The treatment of pneumonias with sulfanilamide. *Illinois med. J.*, LXXIII., 422.

MERTINS, P. S., and MERTINS, P. S., Jr., 1937, Meningitis due to Type IV pneumococcus, with recovery : report of case. *Arch. Otolaryng., Chicago*, XXV., 659.

MILLETT, J., 1937[1], The action of sulfanilamide in a case of Type III pneumococcus pneumonia. *N.Y. St. J. Med.*, XXXVII., 1743.

MILLETT, J., 1937[2], The intrathecal use of prontosil soluble report of a case of Type III pneumococcus meningitis and septicæmia treated with prontosil soluble, with complete autopsy report. *J. Amer. med. Ass.*, CIX., 2138.

NEAL, J. B., JACKSON, H. W., and APPELBAUM, E., 1937, Summary of methods used in treating meningitis secondary to infections of ears and sinuses. *Laryngoscope, Lond.*, XLVII., 317.

NITTI, F., BOVET, D., and DEPIERRE, F., 1937, Action des dérivés du *p*-aminophénylsulfamide (1162F) sur les streptocoques hémolytiques *in vitro*. *C. R. Soc. Biol., Paris*, CXXIV., 16.

OSGOOD, E. E., and BROWNLEE, I. E., 1938, Culture of human marrow. A comparative study of the effects of sulphanilamide and antipneumococcus serum on the course of experimental pneumococcic infections. *Arch. int. Med.*, LXII., 181.

OSTERHOLZ, E., 1938, Untersuchungen zur Chemo- und Serotherapie der experimentellen Pneumokokken-infektion. *Zbl. Bakt.*, Abt. I. Orig., CXLII., 293.

QUERY, R. Z., 1938, Pneumococcus Type VII meningitis treated with sulfanilamide and specific serum. *J. Amer. med. Ass.*, CXI., 1371.

REID, G. C. K., and DYKE, S. C., 1938, Pneumococcal meningitis treated with M. & B. 693 : recovery. *Lancet, ii.*, 619.

ROBERTSON, K., 1938, Case of pneumococcal meningitis treated with M. & B. 693. *Ibid., ii.*, 728.

ROSENTHAL, S. M., 1937, Studies in chemotherapy ; effect of *p*-aminobenzenesulphonamide on pneumococci *in vitro*. *Publ. Hlth. Rep., Wash.*, LII., 192.

ROSENTHAL, S. M., BAUER, H., and BRANHAM, S. E., 1937, Studies in chemotherapy. IV. Comparative studies of sulphonamide compounds in experimental pneumococcus, streptococcus and meningococcus infections. *Ibid,*, LII., 662.

SCHMIDT, L. H., 1937, Use of sulfanilamide in treatment of type XIV pneumococcus infections in mice. *Proc. Soc. exp. Biol., N.Y.*, XXXVII., 205.

TELLING, M., and OLIVER, W. A., 1938, Massive pneumonia, Type III, with massive collapse, treated with 2-(*p*-aminobenzenesulphonamido) pyridine. *Lancet*, *i.*, 1391.

WHITBY, L. E. H., 1937, An experimental assessment of the therapeutic efficacy of amino compounds with special reference to *p*-benzylaminobenzenesulphonamide. *Ibid.*, *i.*, 1517.

WHITBY, L. E. H., 1938, Chemotherapy of pneumococcal and other infections with 2-(*p*-aminobenzenesulphonamido) pyridine. *Ibid.*, *i.*, 1210.

WIEN, R., 1938, The toxicity of 2-(*p*-aminobenzenesulphonamido) pyridine. *Quart. J. Pharm.*, XI., 217.

YOUNG, F., 1938, Pneumococcal meningitis treated with sulphanilamide : recovery. *Brit. Med. J.*, *ii.*, 286.

MENINGOCOCCAL INFECTIONS

Since hæmolytic streptococcal infections react so readily to the sulphonamide group of drugs, it is not surprising that their action should have been tested on other bacteria, among them the meningococcus. Buttle *et al.* (1936) first showed that sulphanilamide protected mice against experimental meningococcal infections, when the organisms were inoculated, following Miller and Castle's method, in a mucin basis. These results were extended and confirmed by Proom (1937), and later by Levaditi and Vaisman (1937), and Rosenthal, Bauer and Branham (1937). It was thus shown that, if given immediately after injection of the organisms, sulphanilamide will protect mice against approximately a million minimal infecting doses of both Groups I and II meningococci. If, however, the administration of the drug is delayed for several hours after injection of the organisms, a much lower degree of protection is obtained. Rosenthal and his colleagues (1937) observed that, while doses of 0·5 gm. per kilogram of body weight of sulphanilamide given to mice infected with meningococci produced 30 per cent. of survivors, *p*-aminobenzenesulphonyl - *p* - aminobenzenesulphonamide (disulphanilamide) was even more effective, since it protected 70 per cent. of mice (*cf.* also Feinstone *et al.*, 1938) : *p*-benzylaminobenzenesulphonamide, however, was found by both Whitby (1937) and Rosenthal *et al.* (1937) to be quite inactive against meningococcal infections, thus showing that the action on streptococci does not necessarily parallel that on meningococci.

The only sulphone derivatives which have been investigated for their action on meningococcal infections in mice are 4 : 4′-diaminodiphenylsulphone and 4 : 4′-diacetyldihydroxydiphenylsulphone. The former compound was regarded by Feinstone *et al.* (1938) as less effective than sulphanilamide. 4 : 4′-Diacetyldihydroxydiphenylsulphone was found by Levaditi, Girard and Vaisman (1938) to protect 73 per cent. of mice when given by mouth in doses of 10 mgm. Levaditi, Vaisman and Reinié (1937) believed that 4-nitro-4-aminodiphenylsulphoxide neutralises the endotoxins of the meningococcus.

Brown (1937) and Branham and Rosenthal (1937) and Branham (1938) studied and compared the action of antimeningococcic sera and sulphanilamide on meningococci of Groups I, II and III. In three strains the drug was the more effective, in four serum, and in three no difference could be detected between the action of sulphanilamide and serum. No correlation could be established between therapeutic response and the serological type or virulence of the organism. A combination of antimeningococcic serum and sulphanilamide was, however, more efficient than either alone, and was in fact greater than the mere additive effect of drug and serum, suggesting that the combination, at any rate in mice, exerted some synergic action.

The experimental observations in mice have been amply confirmed in the treatment of meningococcal meningitis in man, but with the exception of three patients cured by prontosil (Pongratz, 1937), and of a child of three and half months of age who recovered after having received 36 gm. of uleron and no serum (Gehrt, 1938), all the cases have been treated either by sulphanilamide alone or by a combination of sulphanilamide and immune serum. In view of the fact that the majority of cases have received this combination, it is not easy to assess correctly the value of the drug treatment. In a series of 52 cases, however, treated by Schwentker *et al.* (1937) and Schwentker (1937) with a mortality of 15 per cent., comparable serum-treated cases had a mortality of 30 per cent. A 0·8 per cent. solution of sulphanilamide was injected subcutaneously and intrathecally. Eldahl (1938) also employed a 0·8 per cent. solution of sulphanilamide, both intramuscularly and intrathecally. The amount of the solution injected into the spinal canal varied from 5 to 30 c.c. according to the amount of cerebrospinal fluid withdrawn : the amount of the drug injected was, however, always less than the amount of fluid withdrawn. In a series of twelve children under four years of age three died, but the average fatality rate among children of this age in the same hospital for the previous six years had been 70 per cent. Carey (1937) reported five cases successfully treated, while Banks (1938) obtained better results with sulphanilamide alone by mouth than with serum alone or with a combination of serum and sulphanilamide. In the majority of the other cases recorded (Armand Delille *et al.*, 1937 ; Jennings and Southwell-Sander, 1937 ; McIntosh *et al.*, 1937 ; Bernstein, 1937 ; Crean, 1937 ; Crawford and Fleming, 1938 ; Branham, 1938 ; Jewsbury, 1938 ; and Willien, 1938) serum has been combined with sulphanilamide. Certain of the results have been striking. Branham (1938), for instance, had only one death, in a man of fifty, among fifteen patients. Crawford and Fleming (1938) also lost only one of ten children, four of whom were under one year of age : in this series sulphanilamide was given orally and by intramuscular injection. While some workers have stressed the desirability of giving the drug intrathecally, others have pointed out that in any case sulphanilamide readily penetrates into the cerebrospinal fluid and after oral administration the cerebrospinal fluid becomes bacteriostatic to meningococci (Branham and Rosenthal, 1937, and Neter, 1938). Some observers believe that the concentration of sulphanilamide in the cerebrospinal fluid bears no relation to the course of the disease, nor to the speed with which the cerebrospinal fluid is sterilised. Banks (1938), however, states that the concentration in the cerebrospinal fluid should be maintained for at least three days at a concentration of 3 to 5 mgm. per 100 c.c. Until further evidence has accumulated, it would seem wisest to restrict intrathecal medication to those cases in which meningeal symptoms do not clear up readily or which show intolerance to the drug by other routes.

In addition, no final conclusion can yet be reached as to the most satisfactory dose or the length of time for which the drug should be administered. Thus Bernstein (1937) gave 38 gm. in eighteen days to a child of seventeen months, McIntosh *et al.* (1937) 3 gm. in three days, while Crawford and Fleming (1938) gave 2 to 3 gm. a day divided into six doses. Banks (1938) gave children under two 3 gm. daily, adults under forty 10·5 gm., adults over forty 9 gm. daily. Apart from dosage it is essential that treatment should be begun as soon as possible.

Although further observation on many points is necessary there is no doubt that the response to treatment is rapid, some recovered cases having been almost moribund, while the cerebrospinal fluid becomes sterile in from one to three days, sometimes in fact after a single intrathecal injection. Sulphanilamide is equally effective in infections due to meningococci of Groups I and II.

In addition to the actual cases of meningococcal meningitis, Zendel and Greenberg (1937) describe the successful treatment with prontosil and sulphanilamide of two cases of meningococcal septicæmia. Eldahl (1938) cured three children with meningococcal septicæmia by intrathecal and intramuscular injections of sulphanilamide, while Dimson (1938) reports the rapid recovery of a case of chronic meningococcal septicæmia after the administration of 2-(*p*-aminobenzenesulphonamido) pyridine.

Contrary to the negative results in animals, Hannah and Hobson (1938) have recently treated nine cases of meningococcal meningitis with soluseptasine and proseptasine. Seven recovered.

Drugs of the sulphonamide series are thus of undoubted efficiency in meningococcal infections and should be of extreme value in combating both sporadic cases and the widespread epidemics of meningococcal meningitis that occur among the native inhabitants of Central Africa (*cf.* Craddock, 1938). There is, however, some evidence to suggest that, to prevent recurrences, sulphonamide drugs should be administered for some time after the disappearance of clinical symptoms.

References

Armand-Delille, P. F., Lestocquoy, Tiffeneau, R., Wolinetz and Meyer, J., 1937, Chimiothérapie de la méningite cérébrospinale à meningocoque. *Pr. méd.*, XLV., 1515.

Banks, H. S., 1938, Serum and sulphanilamide in acute meningococcal meningitis. *Lancet*, *ii.*, 7.

Bernstein, S. S., 1937, Report on use of sulfanilamide at Children's Hospital at Michigan. *J. Pediat.*, XI., 198.

Branham, S. E., 1938, Serum, antitoxin and drugs in the treatment of meningococcus meningitis. *Med. Ann District of Columbia*, VII., 1.

Branham, S. E., and Rosenthal, S. M., 1937, Studies in chemotherapy. V. Sulphanilamide, serum and combined drug and serum therapy in experimental meningococcus and pneumococcus infections in mice. *Publ. Hlth. Rep.*, *Wash.*, LII., 685.

Brown, M. T., 1937, Protective action of sulfanilamide and antimeningococcus serum on meningococcus infection of mice. *Johns. Hopk. Hosp. Bull.*, LXI., 272.

Buttle, G. A. H., Gray, W. H., and Stephenson, D., 1936, Protection of mice against streptococcal and other infections by *p*-aminobenzenesulphonamide and related substances. *Lancet*, *i.*, 1286.

Carey, B. W., 1937, The use of para-aminobenzenesulfonamide and its derivatives in the treatment of infections due to the streptococcus hæmolyticus, the meningococcus and the gonococcus. *J. Pediat.*, XI., 202.

Craddock, A. L., 1938, Sulphanilamide in meningitis. *Brit. med. J.*, *i.*, 1335.

Crawford, T., and Fleming, G. B., 1938, Treatment of meningococcal meningitis with sulphanilamide. *Lancet*, *i.*, 987.

Crean, T. F., 1937, Use of prontosil in treatment of gonorrhœa. *Ibid.*, *ii.*, 895.

Dimson, S. B., 1938, Chronic meningococcal septicæmia treated with 2-(*p*-aminobenzenesulphonamido) pyridine. *Ibid.*, *ii.*, 424.

Eldahl, A., 1938, Intrathecal sulphanilamide in meningococcal meningitis *Ibid.*, *i.*, 712.

Feinstone, W. H., Bliss, E. A., Ott, E., and Long, P. H., 1938, Observations concerning the toxicity, absorption and therapeutic effect of sulphanilamide and certain related organic sulphur-containing compounds in experimental infections in mice. *Johns Hopk. Hosp. Bull.*, LXII., 565.

Gehrt, J., 1938, Behandlung der Meningitis epidemica mit Uliron. *Dtsch. med. Wschr.*, LXIV., 409.

Hannah, R. H., and Hobson, F. G., 1938, Meningococcal meningitis treated with proseptasine and soluseptasine. *Lancet*, *ii.*, 937.

Jennings, G. H., and Southwell-Sander, G., 1937, Anæmia and agranulocytosis during sulfanilamide therapy. *Ibid.*, *ii.*, 899.

Jewsbury, E. C. O., 1938, The use of sulphanilamide in the treatment of meningococcal meningitis. *Ibid.*, *i.*, 1262.

Levaditi, C., Girard, A., and Vaisman, A., 1938, Chimiothérapie des infections expérimentales (gonocoque, méningocoque, pneumocoque et streptocoque). *C. R. Soc. Biol.*, *Paris*, CXXVII., 19.

Levaditi, C., and Vaisman, A., 1937, Chimiothérapie de l'infection méningococcique expérimentale de la souris. *Ibid.*, CXXV., 604.

Levaditi, C., Vaisman, A., and Reinié, L., 1937, Chimiothérapie antiendotoxique. *Ibid.*, CXXVI., 1093.

McIntosh, R., Wilcox, D. A., and Wright, F. H., 1937, Results of sulfanilamide treatment at Babies Hospital, New York City: survey of 58 cases observed prior to June 10th, 1937. *J. Pediat.*, **XI.**, 167.

Neter, E., 1938, Bacteriostatic action of sulfanilamide upon meningococcus in spinal fluid. *Proc. Soc. exp. Biol., N.Y.*, **XXXVIII.**, 37.

Pongratz, R., 1937, Prontosil bei Meningitis epidemica. *Dtsch. med. Wschr.*, **LXII.**, 1015.

Proom, H., 1937, The therapeutic action of *p*-aminobenzenesulphonamide in meningococcal infection of mice. *Lancet*, *i.*, 16.

Rosenthal, S. M., Bauer, H., and Branham, S. E., 1937, Studies in chemotherapy. IV. Comparative studies of sulfonamide compounds in experimental pneumococcus, streptococcus, and meningococcus infections. *Publ. Hlth. Rep., Wash.*, **LII.**, 662.

Schwentker, F. F., 1937, Discussion on " Observations on the mode of action of sulfanilamide." *J. Amer. med. Ass.*, **CIX.**, 1527.

Schwentker, F. F., Gelman, S., and Long, P. H., 1937, The treatment of meningococcic meningitis with sulfanilamide. *Ibid.*, **CVIII.**, 1407.

Whitby, L. E. H., 1937, An experimental assessment of the therapeutic efficacy of amino compounds with special reference to *p*-benzylaminobenzenesulphonamide. *Lancet*, *i.*, 1517.

Willien, L. J., 1938, Sulfanilamide therapy in meningococcic meningitis. *J. Amer. med. Ass.*, **CX.**, 630.

Zendel, J. F., and Greenberg, D., 1937, Meningococcemia treatment with sulfanilamide and prontosil—report of 2 cases. *N. Y. St. J. Med.*, **XXXVII.**, 1744.

INFECTIONS DUE TO THE GONOCOCCUS

Gonococcal infections have now been treated with sulphanilamide and uleron, to a less extent with prontosil, prontosil soluble, 2(*p*-aminobenzenesulphonamido) pyridine, and in France with di(*p*-acetylaminophenyl) sulphone (1399F or Rodilone). Despite a few early failures, there is general agreement that symptomatic cure of gonorrhœal infections can be readily produced in a high proportion of cases by drugs of the sulphonamide series, but as yet there is considerable uncertainty as to the most suitable compound, the correct moment to initiate treatment, the length of time necessary to continue administration of the drug, the most satisfactory dosage, the value of additional local treatment and the actual percentage of cures. The action of sulphanilamide in gonorrhœa is satisfactorily explained by the fact that when the kidneys are normal the drug is excreted in the urine in concentrations that are bactericidal against the organisms usually found in infections of the urinary tract (Helmholz, 1937). This is specially true when the urine is alkaline. Thus the lower genito-urinary tract is continually in contact with a strongly bactericidal urine, and in addition, as demonstrated experimentally by Farrell, Lyman and Youman (1938), the secretion of the glands, notably the prostate, draining into the posterior urethra, contains sulphonamide in bactericidal concentrations. The chances of infection spreading to the posterior urethra, to the prostate and the epididymis are thus very considerably decreased. In this connection observations on the bactericidal action of sulphanilamide on the gonococcus *in vitro* are of considerable interest. Wengatz, Boak and Carpenter (1938) found that after an exposure of twenty hours, 0·01 per cent. of sulphanilamide caused a decrease in the number of viable organisms, but complete failure to obtain growth in subcultures was not noted till after an exposure of at least fifty hours. When gonococci were subjected to a temperature of 41·5° C. and to 0·01 per cent. of sulphanilamide it was found that the time necessary to cause death was half that required when the drug was not present. Felke (1937 [1, 2]) and also Cohn (1938) found a similarity between the resistance of strains of gonococci to sulphanilamide and uleron *in vitro* and the resistance of the same organisms in patients treated with these drugs, while Levaditi and Vaisman (1937 [2]) observed a certain similarity between the bactericidal power *in vitro* and the curative action *in vivo* of sulphanilamide, uleron and a number of sulphone and sulphoxide compounds, provided that they were all tested against a single strain of gonococcus. The therapeutic action *in vivo* and the bactericidal action *in vitro* did not parallel the virulence of the organisms, nor could any relation be established between bactericidal action *in vitro* and therapeutic action *in vivo* if a single compound were tested against a number of different strains.

The first observations on the clinical treatment of gonorrhœa were made in Germany by Linser (1936), who employed prontosil with somewhat uncertain results : he advised the combination of prontosil with the usual local irrigations, as did Haugk (1936). Prontosil itself appears to be inferior to sulphanilamide, which was employed early in 1937 at the Mayo Clinic by Buchtel and Cook, who treated twenty-one men and ten women : two men and two women failed to respond to treatment. The next observations were published by Dees and Colston (1937) and by Reuter (1937). The former reported observations on forty-seven patients who were given 4·8 gm. daily in four doses for two days, 2·6 gm. daily for three days and 2·4 gm. daily for from four to eight days ; no local treatment was employed. In thirty-six patients the discharge and the gonococci disappeared in under five days, in five the clinical symptoms promptly disappeared but gonococci were still present in the urethra, in three others who at first responded relapses occurred, and three failed to respond in any way to treatment.

Reuter (1937) treated 100 patients, forty with recent and sixty with long-standing infections. Of the former three, of the latter seven, failed to respond to treatment. The average time for clinical recovery was five days, while treatment consisted of 2·6 gm. daily, given in doses of 0·65 gm. after the three principal meals and at bedtime. Following on the appearance of these two papers, an immense number of communications have appeared from all parts of the world dealing with the treatment of gonorrhœal infections by sulphanilamide.

Johnson and Pepper (1937) treated seventy-five patients with daily divided doses of 5 gm. for two or three days, followed by 4 gm. for two to four days, with subsequent reduction to 2 to 3 gm. Of sixty-four patients observed over a sufficiently long period more than half relapsed or failed to respond to treatment, but in only eight of twenty-seven cases did an anterior urethritis spread to the posterior urethra. Two cases of epididymitis and one periurethral abscess developed, however, during the course of treatment and 55 per cent. of the patients developed toxic reactions.

Cokkinis and McElligott (1938) treated 491 men and 142 women. Sulphanilamide was begun eight to ten days after the onset of the disease and continued for three weeks. For the first two weeks 3 to 5 gm. were given daily and for the third week 3 gm. Permanent cure was obtained in 90 per cent. of the male patients. In women the results were less good, owing to their greater liability to minor toxic effects. The incidence of complications was one-seventh to one-eighth what it was before the use of sulphanilamide.

The majority of observers are now agreed that in sulphanilamide there has been found a compound that readily clears up a considerable percentage of cases of gonorrhœa, with a reduction in the number of metastatic complications and in the period of sickness. O'Hanlon (1938), for instance, has shown that the average period of invalidity amongst the troops at Aldershot has been reduced from sixty-two to twenty-one days. A number of observers (Cokkinis, 1937 and 1938 ; Crean, 1937 ; Durel, 1938 ; and Orr, 1937), however, have reported that a certain number of patients, variously estimated at from 10 to 20 per cent., entirely fail to show any sign of improvement. It has therefore been suggested that certain strains are naturally drug resistant, while Felke (1937[1]) claims that it is possible to produce drug-resistant strains of gonococci *in vitro*. It has been noted, however, that in some instances where a strain has proved resistant to sulphanilamide it may rapidly yield to treatment with uleron (*cf.* O'Hanlon, 1938), while it is undoubtedly true that strains of gonococci vary very considerably in their reaction to compounds of the sulphonamide group. It must be remembered that certain cases of gonorrhœa have always proved extraordinarily recalcitrant to any therapeutic measures whatsoever, and before drug resistance, such as is seen in trypanosomes, is invoked as an explanation much further investigation is required.

Although urethral discharges cease and gonococci disappear, evidence goes to show that gonococcal infection may still persist and give rise to chronic prostatis or cystic littritis. Gillies Annan (1938) therefore recommends routine urethroscopy and repeated examination of prostatic smears in all cases, even in the absence of any definite indication for such procedures.

A further question of importance, yet to be decided, is the most suitable time to begin treatment. Cokkinis (1937), for instance, obtained very similar results in both acute and chronic cases. O'Hanlon (1938), Crean (1937) and Hering (1938), on the other hand, emphasised the importance of beginning sulphanilamide treatment as soon as possible, while Herrold (1937) and van Slyke *et al.* (1937) obtained better results after some delay.

Some observers have preferred employing both sulphanilamide and prontosil soluble (*cf.* Crean, 1937, and Hanschell, 1938[1]), while there is general agreement that chemotherapeutic treatment should be combined with irrigation of the urethra. In association with the oral administration of sulphanilamide, not more than 40 c.c. of prontosil soluble (5 per cent. solution) should be given intramuscularly or 15 c.c. intravenously during the course of a week. Hanschell (1938[1]) recommends that large quantities of fluid should be taken by mouth, a procedure which, however, by flushing out the kidneys, tends to decrease the therapeutic efficiency of sulphanilamide. Ballenger, Elder and McDonald (1937) suggest combining thermal with sulphanilamide treatment.

In addition to gonorrhœa in the adult, sulphanilamide has been used with apparent success in cases of vulvovaginitis in children. Carey (1937) treated two cases with favourable results, but the most extensive series is that reported by Hoffmann *et al.* (1938), who treated twenty-five children varying in age from three months to ten years. During the first two days the daily dosage was 0·051 gm. per lb. of body weight, given in four equally divided doses at intervals of six hours. For the next five days 0·04 gm. per lb. was given per day, while during the second and third weeks the daily dose was reduced to 0·024 gm. per lb. At the end of three weeks a rest period of one week was prescribed, then the course was repeated with a second rest period of one week and, if necessary, a third course. It was observed, however, that if two courses failed to bring about cure there was little use in continuing treatment, even though children tolerate sulphanilamide better than adults. Of the twenty-five children thus treated seven were cured in an average of 17·3 days and nine in an average of 42·9 days.

In the complications associated with gonorrhœa, sulphanilamide has proved of considerable value, while its importance in reducing the incidence of complications has already been noted. Branham, Mitchell and Brainin (1938) reported a case of meningitis associated with gonorrhœa which they believed was cured by sulphanilamide, while a similar case has been described by Marven and Wilkinson (1938), though the authors are uncertain whether recovery was actually due to the drug. One case of arthritis and synovitis was cured by sulphanilamide, as were two cases of posterior urethritis (O'Hanlon, 1938). In so-called gonorrhœal rheumatism, Brulé, Hillemand and Vildé (1937) produced a cure in two out of three cases. Prontosil and sulphanilamide have been recommended in ophthalmia neonatorum; treatment, both local and general, has been given with apparent success. Willis (1938) cured four cases with daily doses of from 0·2 to 0·25 gm. per kilogram of body weight of sulphanilamide. In this connection it may be mentioned that Harrie (1938) treated four patients with panopthalmitis, in two of whom the eye was saved, while Fernández and Fernández (1938) recorded the successful treatment of eight patients with gonorrhœal ophthalmia.

In addition to sulphanilamide and prontosil soluble, proseptasine and soluseptasine have been used in France by Durel (1937). The former compound has apparently little action in gonorrhœal infections in the male, but in women with endocervicitis, both gonorrhœal and non-gonorrhœal, it appears to be of value in doses of from 4 to 5 gm. per day. Johnson and Pepper (1937) also found it useless in gonorrhœa in the male, while 50 per cent. of the patients developed toxic reactions. Soluseptasine, given intravenously in doses of 20 c.c. of a 6 per cent. solution a day, was of no value, but the results were better when daily doses of 5 gm. were given by mouth for fifteen to twenty days. Gonococci disappeared in from two to seven days, while the discharge in acute cases ceased in from two to five days. In more chronic cases the morning discharge disappeared in from seven to seventeen days. Jausion (1937), who also used soluseptasine intramuscularly and intravenously, claimed to have cured five of seven cases. In France also 4 : 4′-diacetylaminodiphenylsulphone has been employed to a limited extent in the treatment of gonorrhœa under the name of Rodilone or 1399F (*cf.* chemotherapy of streptococcal infections, p. 373, and pneumococcal infections, p. 385). Boyer, Nitti, Tréfouël and Tréfouël (1937) gave from 2 to 3 gm. a day, *per os* while Palazzoli and Bovet (1937) gave from 3 to 5 gm. daily. When the drug was used alone in acute cases, six failures were encountered among sixteen patients, but the results were better when oral medication was combined with urethral irrigation with 1 in 2,000 protargol, for of 20 cases given 3 gm. daily by mouth 14 were cured, while of 26 cases given 2 gm. by mouth and urethral lavage 22 were cured. In chronic cases the results are said to have been striking, for in 11 out of 12 cases the urine became completely negative within twenty-four hours.

Of special interest in relation to the chemotherapeutic treatment of gonococcal infections is " Uleron " (DB 90 or diseptal A), *p*-aminobenzenesulphonyl-4-aminobenzenesulphonedimethylamide which, together with the monomethylamide (DB 87 or diseptal B) and the non-methylated amide (DB 32, diseptal C, disulphanilamide), were recommended by Domagk (1937) for the treatment of gonorrhœa. Uleron (*cf.* chemotherapeutic treatment of streptococcal, p. 368, and staphylococcal, infections, p. 410) has been principally employed by Felke (1937 [2]), Grütz (1937) and others in Germany.

RESULTS OF TREATMENT OF GONORRHŒA BY ULERON AND DISEPTAL B (Löhe, Schölzke and Zürn, 1938)

Site of Disease.	Case.	Cure by one course.	Relapses.		
			After 1st course.	After 2nd course.	After 3rd course.
Women.					
Urethra alone	10	10	—	—	—
Cervix alone	26	21	5	2	—
Rectum alone	3	3	—	—	—
Urethra, cervix and rectum	38	28	10	4	1
With infection of Bartholin's gland	23	16	7	3	3
Men					
Anterior urethra	6	4	2	1	1
Anterior and posterior urethra—					
Under 4 weeks	21	17	4	2	—
Over 4 weeks	9	8	1	—	—
With prostatitis alone	23	15	8	4	3
With epididymitis alone	27	23	4	2	1
With combined complications of the urethral regions	14	11	3	2	1
Totals	100	78	22	11	6

Grütz (1937), who tested DB 32, 87 and 90, found that of 36 cases, including acute and chronic infections, 24 were cured, 3 relapsed and 9 failed to react to treatment. Felke (1937 [1]) observed that while cases of gonorrhœa in the female reacted very satisfactorily to uleron, very acute cases and those of long standing that had already undergone prolonged treatment were more difficult to cure. When, however, acute cases had been left for a few weeks, treatment was at once followed by prompt disappearance of symptoms. It is suggested, therefore, that the body tissues must become somewhat resistant to the infection before uleron produces its best results. This also explains the good results in gonorrhœa in the female who, as a rule, comes under treatment at a later stage of the disease than does the male. A number of German observers such as Scherber and Domes (1937), therefore, recommend that in the male no treatment, except possibly mild local irrigation, should be initiated for three weeks after the onset of infection in order to allow time for the body defences to be mobilised. This view is also endorsed by Fischer (1937) and Harrison (1938). O'Malley (1938), who found that six of 22 patients remained uninfluenced, emphasises the importance of giving uleron after meals. Löhe, Schölzke and Zürn (1938), who analysed the results of treatment of 200 patients (*cf.* Table), comprising equal numbers of the two sexes, with diseptal B and uleron, found that with the former drug 13 per cent. relapsed, with the latter 48 per cent. Diseptal B is, however, liable to induce cyanosis (Schubert, 1937). The occurrence in association with both uleron and diseptal B of motor palsies (*cf.* p. 431) is, however, a serious drawback to the use of these compounds, but is unlikely to occur if the drugs are not administered for a period of weeks on end and the dosage is not allowed to exceed 3 gm. per day for five consecutive days, each course being followed by a rest period of two to four days and three courses only being given (Hanschell, 1938 [2]). Uleron is of no prophylactic value in gonorrhœa.

2(*p*-Aminobenzenesulphonamido) pyridine, which has been introduced for the treatment of pneumococcal infections (*cf.* p. 386), has been employed by Lloyd, Erskine and Johnson (1938) in the treatment of gonorrhœa, and also by Bowie (1938). The latter treated 30 patients: 23 of them were given 3 gm. daily for from four to seven days, followed by 1·5 gm. for the same period; 7 received only 1·5 gm. daily. A total dosage of from 15 to 25 gm. in most cases brought about a cure. One patient suffered from breathlessness and methæmoglobinæmia, two each from nausea and dizziness, one from headache and two from drowsiness. Michie and Webster (1938) treated two children with gonococcal ophthalmia with success with this compound.

Up to the present there is no consensus of opinion as to which is the most satisfactory compound for the treatment of gonorrhœa. The choice would seem to lie between sulphanilamide, 2-(*p*-aminobenzenesulphonamido) pyridine and uleron. The two former compounds appear to be equal in toxicity and in therapeutic efficiency. Whether they are superior to uleron is at present undecided. German observers appear to favour uleron, while opinion among British workers is divided. Hanschell (1938 [2]) believes that uleron is the more satisfactory, others such as Cokkinis and McElligott (1938) and Walsh (1938) prefer sulphanilamide on the grounds that uleron, while quite as toxic, fails to cure a number of cases despite the fact that its immediate effects are often remarkable. Further controlled observations are obviously necessary before any agreement can be reached as to the best compound for use in gonorrhœa.

Gonococcal Infections in Mice

The experimental study of gonorrhœa has been seriously handicapped by the absence of an experimental animal in which could be induced an infection in any way resembling gonorrhœa in man. Recently Levaditi and Vaisman (1937 [1]) have found that by injecting mucin as with the meningococcus, fatal acute infections may be induced in mice by intraperitoneal inoculations of gonococci. It is, of course, obvious that such conditions in mice bear little or no resemblance to the disease in man, nevertheless their reaction to chemotherapeutic agents is *prima facie* evidence as to whether any particular medicament will or will not have a therapeutic action on gonococci.

Observations on gonococcal infections of mice have shown that they are readily curable by sulphanilamide itself and by a number of sulphonamide derivatives. Thus doses of 10 mgm. of sulphanilamide *per os* to a 20 gm. mouse gave 55 per cent. of survivors, 5 mgm. 0 per cent. : doses of 15 mgm. of uleron 50 per cent. of survivors, 5 mgm. 30 per cent.; 4-nitro-4′-aminodiphenylsulphoxide is particularly effective in mice, the minimal effective dose being at least one-hundredth of the tolerated dose ; single doses of 1, 2 and 5 mgm. produced 40, 50 and 90 per cent. of survivors, while repeated doses of 0·1 to 0·5 mgm. *per os* cured 90 and 100 per cent. of mice (Levaditi, Girard and Vaisman, 1937). Levaditi and Vaisman (1937 [2] and 1938) believe that this same compound *in vivo* when given by mouth neutralises the endotoxins of the gonococcus in from 55 to 60 per cent. of mice (*cf.* also Carpenter *et al.*, 1938). Levaditi and his colleagues (1937) have found that any modification of the structure of 4-nitro-4-aminodiphenylsulphoxide diminishes its therapeutic activity in mice as follows :—

The diamino derivative is five times less active.
The N-methyl derivative is four to five times less active.
The N-benzyl derivative is five to eight times less active.
The diacetylamino derivative is ten times less active.
The N-dimethyl derivative is ten to fifteen times less active.
The aminoacetylamino derivative is fifteen times less active.

References

BALLENGER, E. G., ELDER, O. F., and McDONALD, H. P., 1937, Sulfanilamide and thermotherapy in gonococcic infections—a preliminary report. *J. Amer. med. Ass.*, CIX., 1037.

BOWIE, F. J. T., 1938, Chemotherapy in gonorrhœa. A preliminary report on the use of 2-(*p*-aminobenzenesulphonamido) pyridine M. and B. 693. *Brit. med. J.*, *ii.*, 283.

BOYER, H., NITTI, F., TRÉFOUËL, J., and TRÉFOUËL, Mme., 1937, Note préliminaire sur l'action de la paradiacetylaminodiphénylsulfone (1399F) dans la blennorragie. *Bull. Soc. franç. Derm. Syph.*, XLIV., 1899.

BRANHAM, S. E., MITCHELL, R. H., and BRAININ, W., 1938, Gonococcus meningitis and some of the difficulties encountered in its recognition. *J. Bact.*, XXXV., 35.

BRULÉ, M., HILLEMAND, P., and VILDÉ, L., 1937, Deux cas de rhumatisme gonococcique traité par la para-amino-phényl sulfamide. *Bull. mém. Soc. méd. Hôp., Paris*, LIII., 1641.

BUCHTEL, H. A., and COOK, E. N., 1937, The use of sulfanilamide in treatment of urinary infections. *Proc. Mayo Clin.*, XII., 444.

CAREY, B. W., 1937, The use of para-aminobenzenesulfonamide and its derivatives in the treatment of infections due to the streptococcus hemolyticus, the meningococcus and the gonococcus. *J. Pediat.*, XI., 202.

CARPENTER, C. M., BARBOUR, G. M., and HAWLEY, P. L., 1938, The inactivation of " gonococcal " toxin *in vitro* by sulfanilamide. *J. Bact.*, XXXVI., 280.

COHN, A., 1938, Effect of sulfanilamide on gonococci : experimental study. *Amer. J. Syph.*, XXII., 1.

COKKINIS, A. J., 1937, Treatment of gonorrhœa with oral sulphanilamide : technique, toxic effects and early results in 250 cases. *Brit. med. J.*, *ii.*, 905.

COKKINIS, A. J., 1938, Sulphonamide in genito-urinary (non-gonococcal infections). *Ibid.*, *i.*, 1151.

COKKINIS, A. J., and McELLIGOTT, G. L. M., 1938, Sulphanilamide in gonorrhœa : an analysis of 633 cases. *Lancet*, *ii.*, 355.

CREAN, T. F., 1937, The use of prontosil in the treatment of gonorrhœa. *Ibid.*, *ii.*, 895.

DEES, J. E., and COLSTON, J. A. C., 1937, Sulfanilamide in gonococcic infections. *J. Amer. med. Ass.*, CVIII., 1855.

Domagk, G., 1937, Weitere Untersuchungen über die chemotherapeutische Wirkung sulfonamidhaltiger Verbindungen bei bakteriellen Infektionen. *Klin. Wschr.*, XVI., 1412.

Durel, P., 1937, Recherches sur la chimiothérapie de la gonococcie à l'aide de divers médicaments antibactériens récents. *Bull. Soc. franç. Derm. Spyh.*, XLIV., 1850.

Durel, P., 1938, Action dans la blennorragie du 1162F. *Pr. méd.*, XLVI., 21.

Farrell, J. I., Lyman, Y., and Youman, G. P., 1938, The rationale of sulfanilamide in gonococcic urethritis. *J. Amer. med. Ass.*, CX., 1176.

Felke, H., 1937 [1], Die Wirkung der Sulfonamidverbindungen auf die Erreger der Gonorrhöe. *Klin. Wschr.*, XVII., 13.

Felke, H., 1937 [2], Die Chemotherapie der Gonorrhöe. *Dtsch. med. Wschr.*, LXIII., 1393.

Fernández, L. J., and Fernández, R. F., 1938, Sulfanilamide in gonorrhœal ophthalmia. *Amer. J. Ophthal.*, XXI., 763.

Fischer, C., 1937, Neue Wege in der Gonorrhöebehandlung. *Fortschr. Therap.*, XLIII., 553.

Gillies Annan, W., 1938, Sulphanilamide treatment of gonorrhœa. *Brit. med. J.*, *ii.*, 91.

Grütz, O., 1937, Neue Grundlegung für die Gonorrhöebehandlung. *Münch. med. Wschr.*, LXXXIV., 1201.

Hanschell, H. M., 1938 [1], Clinical observations on the treatment of gonorrhœa in the male with sulphanilamide. *Brit. J. vener. Dis.*, XIV., 18.

Hanschell, H. M., 1938 [2], Chemotherapy of gonorrhœa. *Brit. med. J.*, *ii.*, 375.

Harrie, P. A., 1938, Sulphanilamide in panophthalmitis. *Prescriber*, XXXII., 96.

Harrison, L. W., 1938, When to start sulphanilamide treatment. *Brit. med. J.*, *ii.*, 90.

Haugk, 1936, Über Gonorrhöetherapie unter besonderer Berücksichtigung der Prontosilbehandlung. *Med. Klinik.*, XXXIII., 1121.

Helmholz, H. F., 1937, Bactericidal power of urine after administration of prontylin by mouth. *Proc. Mayo Clin.*, XII., 244.

Hering, E. R., 1938, Results of sulfanilamide treatment of gonorrhea. *Nav. med. Bull., Wash.*, XXXVI., 63.

Herrold, R. D., 1937, Treatment of gonorrhœa and other infections in the urinary tract with sulfanilamide. *Urol. cutan. Rev.*, XLI., 468.

Hoffman, S. J., Schneider, M., Blatt, M. L., and Herrold, R. D., 1938, Sulfanilamide in the treatment of gonorrhœal vulvovaginitis. *J. Amer. med. Ass.*, CX., 1541.

Jausion, H., 1937, Essai de traitement de la blennorragie par les injections intramusculaires et intraveineuses d'un dérivé soluble de la benzylaminophénylsulfamide. *Bull. Soc. franç. Derm. Syph.*, XLIV., 1891.

Johnson, S. H., and Pepper, D. S., 1937, The evaluation and dangers of the treatment of gonorrhœa with derivatives of the sulfonamide-azo dyes. *Wkly. Roster Med. Digest.*, XXXIII., 465.

Levaditi, C., Girard, A., and Vaisman, A., 1937, La toxi-infection gonococcique expérimentale et son traitement chimiothérapique. *Bull. Soc. franç. Derm. Syph.*, XLIV., 1887.

Levaditi, C., Girard, A., Vaisman, A., Ray, A., and Richard, G., 1937, Sur le 4-nitro-4′-aminodiphenylsulfoxyde et son action dans la toxi-infection expérimentale de la souris. *C. R. Acad. Sci., Paris*, CCV., 1018.

Levaditi, C., and Vaisman, A., 1937 [1], La toxi-infection gonococcique expérimentale et son traitement chimiothérapique. *Pr. méd.*, XLV., 1371.

aditi, C., and Vaisman, A., 1937 [2], Chimiothérapie anti-endotoxique. *C. R. Acad. Sci., Paris*, CCV., 1108.

LEVADITI C., and VAISMAN, A., 1938, Relations entre l'efficacité thérapeutique, antigonococcique expérimentale et le pouvoir microbicide des dérivés benzéniques sulfurés. *C. R. Soc. Biol., Paris*, **CXXVII.**, 1428.

LINSER, 1936, Über Gonorrhöetherapie unter besonderer Berücksichtigung der Prontosilbehandlung. *Med. Klinik.*, **XXXII.**, 1120.

LLOYD, V. E., ERSKINE, D., and JOHNSON, A. G., 1938, Chemotherapy of pneumococcal and other infections. *Lancet*, *i.*, 1305.

LÖHE, H., SCHÖLZKE, K., and ZÜRN, D., 1938, Neue Wege in der Gonorrhöebehandlung. *Med. Klinik.*, **XXXIV.**, 11.

MARVEN, H. P., and WILKINSON, W. E., 1938, Gonococcic meningitis : results of treatment with sulfanilamide. *J. Amer. med. Ass.*, **CX.**, 800.

MICHIE, A. M., and WEBSTER, M. H., 1938, Gonococcal ophthalmia treated with 2-(*p*-aminobenzenesulphonamido) pyridine. *Lancet*, *ii.*, 373.

O'HANLON, O. J., 1938, The treatment of gonorrhœa with sulphanilamide. *J. R. Army med. Corps*, **LXX.**, 183.

O'MALLEY, C. K., 1938, A new chemotherapeutic agent in gonorrhœa. *Sth. Afr. med. J.*, **XII.**, 301.

ORR, H., 1937, Sulfanilamide in the treatment of gonorrhœa. *Canad. med. Ass. J.*, **XXXVII.**, 364.

PALAZZOLI, M., and BOVET, O., 1937, Action de la di(paracétylaminophényl) sulfone (1399F) dans les urétrites gonococciques aiguës et chroniques. *Bull. Soc. franç. Derm. Syph.*, **XLIV.**, 1900.

REUTER, F. A., 1937, The use of sulfanilamide in the treatment of gonorrhœa. *Med. Ann. District of Colombia*, **VI.**, 117.

SCHERBER, G., and DOMES, A., 1937, Die Behandlung der Gonorrhöe beider Geschlechter mit dem Uliron G. Domagks in peroraler Darreichung. *Wien. med. Wschr.*, **LXXXVII.**, 1267, 1298 and 1335.

SCHUBERT, M., 1937, Zur Chemotherapie der Gonorrhöe mit Uliron (DB90) und Diseptal (DB87). *Derm. Wschr.*, **CV.**, 1549.

VAN SLYKE, C. J., THAYER, J. D., and MAHONEY, J. F., 1937, Sulphanilamide therapy in gonococcal infections. *Vener. Dis. Inform.*, **XVIII.**, 417.

WALSH, D. F., 1938, Treatment of gonorrhœa with uleron. *Brit. med. J.*, *ii.*, 215.

WENGATZ, H. F., BOAK, R. A., and CARPENTER, C. M., 1938, The bactericidal effect of sulfanilamide on the gonococcus *in vitro*. *J. Bact.*, **XXXV.**, 36.

WILLIS, T., 1938, Sulfanilamide in ophthalmia neonatorum. *Yale J. Biol. Med.*, **X.**, 275.

INFECTIONS DUE TO STAPHYLOCOCCI

There is now general agreement that, though not nearly so effective as against streptococci, both prontosil and, to a somewhat greater extent, sulphanilamide exert some curative action on staphylococcal infections in mice. In staphylococcal infections most of the treated animals infected with 100 lethal doses recover, while death is delayed for two weeks in those infected with 10,000 lethal doses : in streptococcal infections, on the other hand, mice infected with 10,000 lethal doses can be readily cured by sulphanilamide (Buttle, 1937). The curative effect of disulphanilamide (*cf.* p. 367) on staphylococcal infections in mice was investigated by Domagk (1937), who cured eight out of fourteen animals. Mellon *et al.* (1937) and Feinstone *et al.* (1938) also observed some activity on staphylococcal infections in mice. *p*-Aminobenzenesulphonyl-4-aminobenzesulphonedimethylamide (uleron) is believed by Domagk (1937) to be not only as active as sulphanilamide in the treatment of streptococcal infections, but even more effective than sulphanilamide in the treatment of staphylococcal infections in mice ; comparative data, however, are not provided. Mellon *et al.* (1937), on the other hand, failed to demonstrate its curative action in staphylococcal infections either experimentally or clinically, while Feinstone *et al.* (1938) found uleron inferior both to disulphanilamide and sulphanilamide. Buttle *et al.* (1938) noted that only small infective doses containing about 50,000 cocci could be successfully treated. The same observers showed, however, that the benzylidene derivative of 4 : 4′-diaminodiphenylsulphone was almost equal to sulphanilamide in its curative action on staphylococcal infections in mice. Nitti, Bovet and Hamon (1938) claim that di-(*p*-formylaminophenyl) sulphone and di-(*p*-propionylphenyl) sulphone are active against staphylococci, whereas di-(*p*-acetylaminophenyl) sulphone and di-(*p*-butylaminophenyl) sulphone are quite inactive.

Feinstone *et al.* (1938) found that 4 : 4′-diaminodiphenyl sulphone and di-(*p*-acetylaminophenyl) sulphone saved a third of the mice infected with staphylococci.

Colebrook and Purdie (1937) have reported clinical results which indicate that sulphanilamide may produce some therapeutic effect in cases of staphylococcal septicæmia. Bloch and Pacella (1938) also cured an infant of seventeen days who was suffering from staphylococcal meningitis. A total of 10 gm. of sulphanilamide was given orally and intrathecally over a period of twenty-seven days : the cerebrospinal fluid became sterile after eleven days of treatment. The action of prontosil and sulphanilamide in the treatment of boils, furuncles and staphylococcal abscesses still remains uncertain for, although good results have been obtained by some observers, others have failed to notice improvement. Possibly the combination of immune serum with sulphanilamide may produce more satisfactory results.

Marcus (1938), in the treatment of staphylococcal infections of the skin, gives from 1·5 to 2 gm. of sulphanilamide a day by mouth. If improvement is not shown within a day or two no benefit is likely to occur with larger or long-continued doses. The results obtained by sulphanilamide treatment in a variety of staphylococcal skin infections are shown in the table.

SULPHANILAMIDE IN STAPHYLOCOCCAL SKIN INFECTIONS
(Marcus, 1938)

Condition.	No. of cases.	Results.	
		Good.	Failed or doubtful.
Boils (including two of upper lip)	22	18	4
Bockhart's impetigo	3	0	3
Furuncles	6	6	0
Styes	6	4	2
Carbuncles	6	5	1
Whitlows (acute, 3 ; chronic, 3)	6	6	0
Infections of hand and foot	4	2	2
Subcutaneous abscesses	3	3	0
Abscess of breast	2	0	2
Sycosis barbæ	3	0	3
Acne pustulosa	1	0	1
Total	62	44	18

Reuter (1938), in infections of the genito-urinary tract, found that staphylococci reacted less readily than *Bact. coli* to sulphanilamide by mouth, others however have reported that the results of prontosil treatment in staphylococcal pyelitis and cystitis were almost always satisfactory. Klose (1936) also successfully treated a man with pyelitis and an acute staphylococcal urethritis.

Brown (1938) used sulphanilamide and proseptasine in 35 cases of mastitis, the dose being 2 to 3 gm. daily. *Staphylococcus aureus* was isolated in all cases. The average duration of treatment was five days and no case of mastitis thus treated went on to form a breast abscess. In the treatment of breast abscess, although incision was sometimes necessary, healing appeared to be more rapid than before the use of sulphonamide drugs. Fenton and Hodgkiss (1938) report the cure of a case of staphylococcal septicæmia with 2-(*p*-aminobenzenesulphonamido) pyridine.

References

BLOCH, E., and PACELLA, B. L., 1938, Staphylococcic meningitis. *J. Amer. med. Ass.*, **CX.**, 508.

BROWN, D. B., 1938, Treatment of puerperal sepsis by prontosil and allied compounds. *J. Obst. Gynœc. Brit. Emp.*, **XLV.**, 495.

BUTTLE, G. A. H., 1937, Discussion on the treatment of bacterial diseases with substances related to sulphanilamide. *Proc. R. Soc. Med.*, **XXXI.**, 154.

BUTTLE, G. A. H., DEWING, T., FOSTER, G. E., GRAY, W. H., SMITH, S., and STEPHENSON, D., 1938, The action of substances allied to 4 : 4′-diaminodiphenylsulphone in streptococcal and other infections in mice. *Biochem. J.*, **XXXII.**, 1101.

COLEBROOK, L., and PURDIE, A. W., 1937, Treatment of 106 cases of puerperal fever by sulphanilamide (streptocide). *Lancet*, *ii.*, 1237 and 1291.

DOMAGK, G., 1937, Weitere Untersuchungen über die chemotherapeutische Wirkung sulfanamidhaltiger Verbindungen bei bakteriellen Infektionen. *Klin. Wschr.*, **XLI.**, 1412.

FEINSTONE, W. H., BLISS, E. A., OTT, E., and LONG, P. H., 1938, Observations concerning the toxicity, absorption and therapeutic effect of sulphanilamide and certain related organic sulphur-containing compounds in experimental infections in mice. *Bull. Johns Hopk. Hosp.*, **LXII.**, 565.

FENTON, W. J., and HODGKISS, F., 1938, Staphylococcal septicæmia treated by M. & B. 693. *Lancet*, *ii.*, 667.

KLOSE, G., 1936, Prontosil bei Kokkeninfektion der Harnwege. *Dtsch. med. Wschr.*, **LXII.**, 898.

MARCUS, M., 1938, Sulphanilamide in staphylococcal skin infections. *Brit. med. J.*, *ii.*, 92.

MELLON, R. R., SHINN, L. E., and MCBROOM, J., 1937, Therapy of experimental staphylococcus infections with sulphonamide compounds. *Proc. Soc. exp. Biol., N.Y.*, **XXXVII.**, 563.

NITTI, F., BOVET, D., and HAMON, V., 1938, Le sort des aminophénylsulfones dans l'organisme et leur activité antimicrobienne. *C. R. Soc. Biol. Paris*, **CXXVIII.**, 26.

REUTER, F. A., 1938, The use of sulfanilamide in genito-urinary surgery. *Med. Ann. District of Columbia*, **VII.**, 8.

BRUCELLA INFECTIONS

The value of treatment of brucella infections is often very difficult to assess owing to the sudden spontaneous cures that may occur, nevertheless sufficient evidence is now available to show that such infections are more readily terminated by the administration of compounds of the sulphonamide group than by any other therapeutic agents. The first observations on brucella infections were made in France by Grouès (1936) and Thévenet (1936), who treated cases due to *Brucella melitensis* with rubiazol, while Béthoux, Gourdon and Rochedix (1937) employed prontosil. Later in Germany cases due to *Br. abortus* were cured by prontosil by Suchier (1937), Berger and Schnetz (1937) and Ahringsmann (1937), while in England cures due either to sulphanilamide or to prontosil soluble were reported by Richardson (1938), Francis (1938), Lloyd (1938) and Page (1938). In the last-named author's cases the temperature fell slowly but in the others the fall in temperature was very dramatic. In America, Stern and Blake (1938) and Blumgart (1938) have used sulphanilamide with success. In the latter's case the blood culture was negative three days after the fall of temperature, but in the case reported by Béthoux *et al.* (1938) bacteria were still present in the blood a week after clinical recovery. Tanguy (1938) also found that the organisms did not disappear from the blood stream till some days after beginning treatment.

The most extensive series of cases is that reported from Malta by Neumann (1938). Twenty patients with *Br. melitensis* infections were treated. Four were given 5 c.c. injections of prontosil on alternate days. In only one was the period of pyrexia appreciably shortened. The dosage was almost certainly inadequate. Of sixteen patients given sulphanilamide by mouth fifteen improved, the average duration of fever after the beginning of treatment being seven days.

Experiments on brucella infections in animals have been reported by Montgomerie (1938), who has found that in guinea-pigs given 1·8 gm. of sulphanilamide in twelve days the infection is as a rule eliminated, as is udder infections in goats. Chinn (1938) also found that in guinea-pigs infected with *Br. abortus* and *Br. suis* the organisms can be destroyed by doses of 100 mgm. a day by mouth. *In vitro* experiments on the bacteriostatic action of sulphanilamide on *Br. abortus* are contradictory. Francis (1938) believes that *Br. abortus* is more susceptible to the action of sulphanilamide *in vitro* than *Str. pyogenes* under the same conditions, while Britton (1938) reports that sulphanilamide and other allied compounds show no bacteriostatic action on *Br. abortus*. Chinn (1938), however, with sulphanilamide has obtained a bacteriostatic and a bactericidal action on *Br. abortus, Br. suis* and *Br. melitensis*.

References

AHRINGSMANN, H., 1937, Heilung der Bangschen Krankheit durch Prontosil. *Münch. med. Wschr.*, LXXXIV., 1778.

BERGER, W., and SCHNETZ, H., 1937, Ein Behandlungserfolg bei Morbus Bang mit Prontosil. *Med. Klinik.*, XXXIII., 594.

BÉTHOUX, L., GOURDON, E., and ROCHEDIX, J., 1937, Mélitococcie traitée par le chlorhydrate de sulfaminochrysoïdine, amélioration clinique, persistance des germes dans le sang. *Bull. Mém. Soc. méd. Hôp. Paris*, LIII., 678.

BLUMGART, H. L., 1938, Recovery of a patient with undulant fever treated with sulfanilamide. *J. Amer. med. Ass.*, CXI., 521.

BRITTON, C. J. C., 1938, The effect of sulphonamide compounds on certain bacteria *in vitro*. *Brit. J. exp. Path.*, XIX., 140.

CHINN, B. D., 1938, *In vitro* and *in vivo* effect of sulfanilamide on *Brucella abortus* and *Brucella suis*. *Proc. Soc. exp. Biol., N.Y.*, XXXVIII., 732.

FRANCIS, A. E., 1938, Sulfanilamide in the treatment of undulant fever. *Lancet, i.*, 496.

GROUÈS, P., 1936, Sur quelques cas de mélitococcie traités par le rubiazol seul ou associé à des agents thérapeutiques spécifiques de l'affection. *Lyon méd.*, CLVIII., 615.

LLOYD, J. H., 1938, Sulphonamide-P in *Brucella abortus* infection. *Brit. med. J., i.*, 145.

MONTGOMERIE, R. F., 1938, Sulphanilamide in *Br. abortus* infection in animals. *Vet. Rec.*, L., 380.

NEUMANN, C. Z., 1938, Treatment of undulant fever with prontosil. *Brit. med. J., ii.*, 342.

PAGE, W. A., 1938, *Br. abortus* infection treated by sulphanilamide. *Ibid., i.*, 595.

RICHARDSON, L. A., 1938, Infection with *Brucella abortus* treated with prontosil. *Lancet, i.*, 495.

STERN, R. L., and BLAKE, K. W., 1938, Undulant fever: its treatment with sulfanilamide. *J. Amer. med. Ass.*, CX., 1550.

SUCHIER, W., 1937, Zur Behandlung der Bangschen Krankheit. *Fortschr. Ther.*, V., 305.

TANGUY, Y., 1938, Fièvre de Malte guérie cliniquement avec le traitement trypaflavine, 1162F. Persistance momentanée de micrococques dans le sang. *Bull. Soc. Path. exot.*, XXI., 360.

THÉVENET, V., 1936, Un cas de mélitococcie traité par le rubiazol (*per os*). Terminaison favorable relativement rapide de la maladie. *Lyon méd.*, CLVIII., 668.

INFECTIONS DUE TO *BACTERIUM COLI*

Since prontosil and sulphanilamide are known to be excreted in the urine their effect on bacterial infections of the urogenital tract, especially those due to *Bacterium coli*, has received considerable attention. The first reported observations were those of Temming (1935), in Germany, who treated three infants suffering from pyelitis with prontosil. The results were remarkable, for in all cases the temperature fell, the urine became free from leucocytes and albumen and the general condition rapidly improved. These findings in infants and young children were quickly confirmed by Maraun (1936), Huber (1936), Pernice (1936) and Klein (1936), and were later extended to adults by Klose (1936), Meissner (1937), and Türk (1937). Weiser (1937) also reported the treatment of coccal and mixed infections of the urinary tract with somewhat inconclusive results. Experimentally it was shown by Buttle *et al.* (1937) that in mice sulphanilamide acts curatively in infections due to *Bacterium typhosus*, and has a considerable effect in delaying the death of animals infected with *Bacterium aertrycke* and other members of the genus *Bacterium* such as Friedlander's bacillus.

In Great Britain, sulphanilamide has been used in the treatment of infections of the genito-urinary tract practically to the exclusion of prontosil. There is, however, no reason to think that prontosil is in any way inferior to sulphanilamide in this respect. Kenny, Johnston and von Haebler (1937) treated forty-six patients suffering from *Bact. coli* urinary infections, the daily dose of sulphanilamide given for from five to seven days being from 1·5 to 1·8 gm.: in all cases the temperature and pulse rate were normal within forty-eight hours of the beginning of treatment and the urine became free from pus and albumen in from two to five days. Among sixteen patients with pyelitis of pregnancy, five showed evidence of pre-eclamptic toxæmia, but sulphanilamide in no way aggravated the condition. There were no clinical relapses among these sixteen patients, but in six cases symptomless bacteriuria was subsequently detected. Of seventeen patients with acute pyelitis of the puerperium only one developed a clinical relapse. In chronic pyelitis also the urine rapidly became sterile, although it was noted that in mixed infections with *Proteus vulgaris* this organism was more resistant than *Bact. coli*, while in two patients mandelic acid had previously failed to clear up the infection. In one patient with an atypical *Bact. coli* urinary infection, sulphanilamide had no effect in sterilising the urine, but neither had any other known form of treatment. An interesting finding was that while the urine rapidly became bacteriostatic to most strains of *Bact. coli*, many strains which had yielded readily to treatment *in vivo* were nevertheless comparatively resistant *in vitro*. Cokkinis (1938), in a series of non-gonococcal infections, chiefly *Bact. coli*, of the genito-urinary tract, employed not only sulphanilamide and prontosil soluble, but, in addition, proseptasine, soluseptasine and uleron. Of primary genital infections, twenty-six cases of urethritis showed only three failures, while in four cases of epididymitis there was one failure. One case of an infection with *Streptococcus viridans* was cured by 35 gm. of sulphanilamide in ten days. Among nine cases of acute or subacute pyelitis and three cases of acute cystitis there was only one failure owing to the toxic results produced, but in more than a quarter of the cases organisms reappeared in the urine. Of thirteen infections of the bladder, secondary either to senile enlargement of the prostate or to urethral stricture and infected with *Bact. coli*, *Proteus* and streptococci, four failures were noted.

Barr (1938), who treated sixty-four cases of pyelitis of pregnancy, found sulphanilamide preferable to prontosil soluble. Simple cases were cured in four to five days, but renal and toxic pyelitis required on an average fourteen days to sterilise the urine.

In America, Villani (1937) described the improvement of seven cases of pyelitis with sulphanilamide. The drug, as Kenny and her co-workers (1937) had also found, was effective over a wide *p*H range of the urine. Reuter (1938) found that sulphanilamide is very active on *Bact. coli. Proteus vulgaris* and *Bact. aerogenes*, less active on *Staphylococcus aureus*, and practically inert on *Streptococcus fœcalis*. Its value lies not only in its therapeutic but also in its prophylactic use in cases requiring surgical intervention in the urinary tract, more especially where, as so often happens, chronic prostatitis is associated with cystitis. As a result of these numerous observations, and more especially the investigations of Helmholz (1937 [1 & 2]), it is possible to define with some precision the conditions necessary for success in the treatment of genito-urinary infections by drugs of the sulphonamide group. To obtain the best results a concentration of between 20 and 30 mgm. of sulphanilamide per 100 c.c. of urine is necessary. Although the drug is active over a wide range of *p*H values from 5 to 8, the most satisfactory result is obtained when the *p*H lies between 7 and 8 (Helmholz and Osterberg, 1937, and Mellon and Shinn, 1937). Actually, however, the urine excreted during treatment is alkaline, though there is doubt as to whether this is because sulphanilamide interferes with the tubular reabsorption of bicarbonate and bases. Sulphanilamide appears to exert a bactericidal action on *Bact. coli* throughout the urinary tract, but this effect, according to Mellon and Shinn (1938), is a potentiation of the minimal effect of the urine itself on *Bact. coli*. In its bactericidal action sulphanilamide thus differs greatly from mandelic acid (*cf.* p. 474) which cannot be administered where there is renal damage, but only when the urine is highly acid. Since the effect of *Proteus* is almost invariably to cause an alkaline urine, sulphanilamide is obviously the drug of choice for this infection. *Str. fœcalis* is unaffected by sulphanilamide but susceptible to mandelic acid. In addition, sulphanilamide can be given during the acute stage, but as Buchtel and Cook (1937) and Cokkinis (1938) have emphasised, sulphanilamide is far more toxic than mandelic acid and in operation cases prostration, gastric irritation and cyanosis are especially undesirable. Buchtel and Cook, however, find that when prostatitis is present mandelic acid sterilises the prostatic secretion in only 50 per cent. of cases whereas sulphanilamide, which can actually be demonstrated in the secretion, sterilises just over 95 per cent. of cases. The value as urinary antiseptics of other drugs of the sulphonamide group has yet to be accurately gauged. According to Cokkinis (1938), proseptasine and soluseptasine, though of low toxicity, have a poor bactericidal action : prontosil soluble is useful in operation cases owing to its lack of toxicity, but to obtain an effect equal to that of sulphanilamide from 30 to 50 c.c. of the 5 per cent. solution must be given daily. Uleron, though a little less active than sulphanilamide, causes no prostration, and its toxic effects on motor nerves are avoided if not more than 3 gm. are given daily for not more than five days at a time.

The effect of the sulphonamide group of drugs on *Bact. coli* infections other than those of the urogenital tract has received little attention. Nader (1937), however, treated two cases of meningitis due to *Bact. coli* in children : one recovered. Ottenberg and Berck (1938) obtained cure of a case of pylephlebitis and liver abscess.

References

BARR, A., 1938, Para-aminobenzene sulphonamide in treatment of pyelitis of pregnancy. *Glasgow med. J.*, XII., 18.

BUCHTEL, H. A., and COOK, E. N., 1937, Use of sulfanilamide in treatment of urinary infections. *Proc. Mayo Clin.*, XII., 444.

BUTTLE, G. A. H., PARISH, H. J., MCLEOD, M., and STEPHENSON, D., 1937, The chemotherapy of typhoid and some other non-streptococcal infections. *Lancet*, *i.*, 681.

COKKINIS, A. J., 1938, Sulphonamide in genito-urinary (non-gonococcal) infections. *Brit. med. J.*, *i.*, 1151.

HELMHOLZ, H. F., 1937[1], Bacterial power of the urine after the administration of prontylin by mouth. *Proc. Mayo Clin.*, XII., 244.

HELMHOLZ, H. F., 1937[2], A comparison of mandelic acid and sulfanilamide as urinary antiseptics. *J. Amer. med. Ass.*, CIX., 1039.

HELMHOLZ, H. F., and OSTERBERG, A. E., 1937, Rate of excretion and bactericidal power of sulfanilamide (prontylin) in urine. *Proc. Mayo Clin.*, XII., 377.

HUBER, H. B., 1936, Über Prontosil bei der Behandlung der kindlichen Pyurie. *Münch. med. Wschr.*, LXXXIII., 2014.

KENNY, M., JOHNSTON, F. D., and VON HAEBLER, T., 1937, *p*-aminobenzenesulphonamide in treatment of *Bacterium coli* infections of the urinary tract. *Lancet*, *ii.*, 119.

KLEIN, E., 1936, Prontosil in der Kinderpraxis. *Med. Klinik.*, XXXII., 940.

KLOSE, G., 1936, Prontosil bei Kokkeninfektion der Harnwege. *Dtsch. med. Wschr.*, LXII., 898.

MARAUN, L., 1936, Zur Prontosilbehandlung des Erysipels und der Pyurie. *Kinderärtzl. Prax.*, X., 445.

MEISSNER, W., 1937, Erfahrungen mit Prontosil bei der Behandlung von entzündlichen Erkrankungen der ableitenden Harnwege. *Med. Klinik.*, XXXV., 95.

Mellon, R. R., and Shinn, L. E., 1937, Potentiating influence of urine on sulfanilamide's bacteriostatic effect on *E. coli in vitro*. *Proc. Soc. exp. Biol., N.Y.*, XXXVII., 331.

Mellon, R. R., and Shinn, L. E., 1938, Limiting factors of sulfanilamide's action and the phenomenon of potentiation. *J. Bact.*, XXXV., 36.

Nader, A., 1937, Blut-liquorskranke und Liquortherapie. *Kinderärztl. Prax.*, VIII., 14.

Ottenberg, R., and Berck, M., 1938, Sulfanilamide therapy for suppurative pylephlebitis and liver abscesses. *J. Amer. med. Ass.*, CXI., 1374.

Pernice, W., 1936, Über die Behandlung der kindlichen Pyurie mit Prontosil. *Ibid.*, VII., 304.

Reuter, F. A., 1938, The use of sulfanilamide in genito-urinary surgery. *Med. Ann. District of Colombia*, VII., 8.

Temming, H., 1935, Über Prontosil bei Pyurie. *Kinderärtzl. Prax.*, VI., 400.

Türk, H., 1937, Die Behandlung der Kolipyelozystitis mit Prontosil. *Münch. med. Wschr.*, LXXXIV., 1259.

Villani, A. J., 1937, Treatment of pyelitis with sulfanilamide. *W. Va. med. J.*, XXXIII., 533.

Weiser, A., 1937, Prontosil bei Infektionen der Harnwege. *Med. Klinik.*, XXXV., 674.

INFECTIONS WITH *BACTERIUM TYPHOSUM*

In mice it has been found by Buttle *et al.* (1937) that sulphanilamide protects against multiple lethal doses of *Bacterium typhosum* and *Bact. paratyphosum* B, although the action is not comparable with that exerted on hæmolytic streptococci. Typhoid infections in man, however, differ considerably from those in mice, and up to the present the only clinical trial is that reported by Schmidt (1936), who employed prontosil with what is claimed to be success in three cases. Subbarao (1938) reports rapid improvement in a patient suffering from typhoid fever after treatment with sulphanilamide. Others have obtained negative results. Barer (1937) recorded a case of typhoid pyelitis and bacilluria which had first been treated by hexamine and ammonium mandelate without improvement. After the patient had received 0·5 gm. of sulphanilamide by mouth twice daily for five days, the urine became sterile but was again infective on the ninth day. Thereafter the same dose of sulphanilamide was repeated for fourteen days and the urine appeared to become permanently sterile. No bactericidal or bacteriostatic effect of sulphanilamide and related drugs could be found by Britton (1938) on *Bact. typhosum*, *Bact. paratyphosum* A and B, *Bact. enteritidis*, *Bact. aertrycke* and *Bact. dysenteriæ*, Flexner, Shiga and Sonne.

Further observations are required to determine the value of sulphanilamide and its allies on typhoid and dysenteric infections in man.

References

BARER, M., 1937, Typhoid bacilluria treated with sulphanilamide. *Lancet*, *ii.*, 964.

BRITTON, C. J. C., 1938, The effect of sulphonamide compounds on certain bacteria *in vitro*. *Brit. J. exp. Path.*, XIX., 140.

BUTTLE, G. A. H., PARISH, H. J., McLEOD, M., and STEPHENSON, D., 1937, The chemotherapy of typhoid and some other non-streptococcal infections in mice. *Lancet*, *i.*, 681.

SCHMIDT, J., 1936, Ein Beitrag zur Prontosilbehandlung bei septischen und ähnlichen Erkrankungen. *Münch. med. Wschr.*, LXXXIII., 2122.

SUBBARAO, K. V., 1938, Treatment of typhoid fever with sulphanilamide. *Brit. med. J.*, *ii.*, 638.

ACTINOMYCOSIS

Up to the present very few observations have been made on the treatment of infections due to the higher bacteria. Recently, however, Walker (1938) has recorded the effect of administering sulphanilamide to a patient suffering from actinomycosis. Treatment consisted of 1 gm. of sulphanilamide by mouth for one week and resulted in rapid lessening of the discharge and disappearance of the typical sulphur granules.

Reference

WALKER, O., 1938, Sulphanilamide in the treatment of actinomycosis. *Lancet*, *i.*, 1219.

INFECTIONS DUE TO GAS GANGRENE BACILLI

Only one clinical report has been published of the treatment of gas gangrene infection in man, Bohlman (1937) having successfully treated three cases suffering from infection with *Clostridium welchii*, acquired despite the prophylactic injection of antitoxin. In two cases the temperature fell to normal on the second day of treatment, and the toxic symptoms disappeared, but recovery was complicated by the development of osteomyelitis. In the third case the temperature did not fall till the seventh day and was not normal till the twenty-sixth day of illness.

The only experimental observations carried out in animals have been those of Bliss and Long (1937) and Long and Bliss (1937), who found that sulphanilamide protected mice against infection with *Cl. welchii.* Domagk (1937) obtained similar results with uleron. Natvig (1938), however, obtained negative results in gas gangrene infections in mice treated with sulphanilamide. *In vitro* Bliss and Long (1937) reported that sulphanilamide has a bacteriostatic action on *Cl. welchii.* Spray (1938) observed that sulphanilamide and prontosil soluble (2·5 per cent. solution) exert a direct bacteriostatic effect up to a dilution of 1 in 1,000 on *Cl. tetani, Cl. lentoputrescens, Cl. novyi, Cl. septicum* and *Cl. histolyticum :* disulphanilamide had a similar action and, in addition, was bacteriostatic up to a dilution of 1 in 125 on *Cl. welchii, Cl. tertium, Cl. sporogenes, Cl. bifermentans* and *Cl. botulinum* Type A. Britton (1938), on the other hand, could detect no bactericidal effect on *Cl. welchii* of sulphanilamide, proseptasine, soluseptasine and sulphonamide derivatives (T 607 and T 626).

Sulphonamide drugs should obviously be tested in every available case of gas gangrene infection.

References

BLISS, E. A., and LONG, P. H., 1937, Observations on the mode of action of sulfanilamide. *J. Amer. med. Ass.*, CIX., 1524.

BOHLMAN, H. R., 1937, Gas gangrene treated with sulfanilamide. Report of three cases. *Ibid.*, CIX., 254.

BRITTON, C. J. C., 1938, The effect of sulphonamide compounds on certain bacteria *in vitro*. *Brit. J. exp. Path.*, XIX., 140.

DOMAGK, G., 1937, Weitere Untersuchungen über die chemotherapeutische Wirkung sulfonamidhaltiger Verbindungen bei bakteriellen Infektionen. *Klin. Wschr.*, XVI., 1412.

LONG, P. H., and BLISS, E. A., 1937, Observations upon the experimental and clinical use of sulphanilamide in treatment of certain infections. *Canad. med. Ass. J.*, XXXVII., 457.

NATVIG, H., 1938, Eksperimentelle undersøkelser over virkningen av prontosil ved gassødeminfeksjoner. *Norsk. Mag. Laegevid.*, XCIX., 1631.

SPRAY, R. S., 1938, Bacteriostatic action of prontosil soluble, sulfanilamide and disulfanilamide on the sporulating anaerobes commonly causally associated with gaseous gangrene. *J. Lab. clin. Med.*, XXIII., 609.

PASTEURELLA INFECTIONS

Comparatively few observations have been made on the effect of sulphonamide drugs on the Pasteurella group of organisms.

Buttle, Parish, McLeod and Stephenson (1937) found, however, that in animals sulphanilamide had a temporary and very slight beneficial effect against *Pasteurella pseudotuberculosis* and *Past. septica*. Against *Past. avicida* Levaditi and Reinié (1938) have found that the following compounds have a slight action both in mice and hens: sulphanilamide, 4-nitro-4′-aminodiphenylsulphone, 4-sulphamido-2′ : 4′-diaminoazobenzene and 4-sulphamido-benzeneazo-3′ : 5′-diaminobenzoic acid.

Of more importance are the observations reported by Carman (1938) on the treatment of bubonic plague. Prontosil given in the form of intramuscular injections of 2·5 c.c. twice a day was apparently responsible for curing three out of six patients. Nine controls all died.

References

BUTTLE, G. A. H., PARISH, H. J., McLEOD, M., and STEPHENSON, D., 1937, The chemotherapy of typhoid and some other non-streptococcal infections in mice. *Lancet*, *i.*, 681.

CARMAN, J. A., 1938, Prontosil in the treatment of oriental plague. *E. Afr. med. J.*, XIV., 362.

LEVADITI, C., and REINIÉ, L., 1938, Chimiothérapie de l'infection determinée chez la souris et la poule par la *Pasteurella avicida*. *C. R. Soc. Biol., Paris*, CXXVII., 1179.

INFECTIONS DUE TO DUCREY'S BACILLUS

The treatment of chancroid has long proved unsatisfactory. Hanschell (1938), however, has described the cure of 20 cases. The patients were given 3 gm. of sulphanilamide a day in divided doses, and in addition received one injection of 5 c.c. and one or two further injections of 10 c.c. of prontosil soluble at three-day intervals. The ulcers healed very rapidly and the buboes subsided in from five to ten days. Similar results have been obtained by Hutchison (1938), who treated eleven patients with sulphanilamide and noted that the average stay in hospital was 15·5 days as against 46·7 days for vaccine treatment and 46·2 for local antiseptic applications. Batchelor and Lees (1938) also treated ten patients, males receiving 8 gm. of sulphanilamide for two days, followed by 6 gm. for four days, while females were given 6 gm. for two days and 4 gm. for four days. Treatment did not appear to interfere with Reenstierna's test.

In America, Kornblith *et al.* (1938) record the successful treatment of 45 cases with sulphanilamide. For the first five days 5·2 gm. were given daily in divided doses and 2·6 gm. daily for nine additional days. Lesions in all cases healed up within two weeks.

References

BATCHELOR, R. C. L., and LEES, R., 1938, Sulphanilamide for chancroid. *Brit. med. J.*, *i.*, 1100.

HANSCHELL, H. M., 1938, Sulfanilamide in the treatment of chancroid. *Lancet*, *i.*, 886.

HUTCHISON, A., 1938, Treatment of bubo with sulphanilamide. *Ibid.*, *i.*, 1047.

KORNBLITH, B. A., JACOBY, A., and WISHENGRAD, M., 1938, Treatment of chancroid with sulfanilamide. *J. Amer. med. Ass.*, CXI., 523.

SULPHONAMIDE DRUGS IN OTHER BACTERIAL INFECTIONS

In addition to the bacteria already discussed, sulphanilamide and prontosil have been tested for therapeutic action, either experimentally in animals or in the treatment of a number of other infections in man. Cokkinis (1938[1]), for instance, in dealing with infections of the genito-urinary tract, found that mixed infections of the bladder due to *Proteus vulgaris*, *Bact. aerogenes* and diphtheroids and associated with senile enlargement of the prostate occasionally cleared up readily.

On the other hand, Gross, Cooper and Lewis (1938) reported that sulphanilamide, 4 : 4′-diacetylamino-diphenylsulphone and 4 : 4′-diaminobenzenesulphonamide have no therapeutic action in mice infected intraperitoneally with *Hæmophilus pertussis.* Cruickshank (1938) also failed with sulphanilamide and with 2-(*p*-aminobenzenesulphonamido) pyridine.

In mice infected with a strain of *Hæmophilus influenzæ* Povitsky (1937) claimed to have obtained a curative action with immune serum and prontosil, reducing the 100 per cent. mortality of the controls to 33 or even 13 per cent. Although Long and Bliss (1937) found that sulphanilamide in a concentration of 1 in 1,000 in serum broth inhibited the growth of *H. influenzæ*, they had no success in the treatment of influenzal pneumonia or meningitis. Similar unsuccessful results in influenzal meningitis in man were reported by Taylor (1938) and McIntosh *et al.* (1937), although success is claimed by Jones (1937) and by Young and Moore (1938). Teggart (1938) obtained a cure of influenzal meningitis with soluseptasine, 3 gm. being given every eight hours for seven days. The temperature became normal after four days' treatment. Albright, Dienes and Sulkowitch (1938) also claim to have brought about cure with sulphanilamide in two cases of nephrocalcinosis in which Gram-negative bacilli, resembling *H. influenzæ*, were present in an alkaline urine.

Reference has already been made to the action of sulphanilamide in infections of the genito-urinary tract due to *Proteus vulgaris*. Basman and Perley (1937) report recovery of a patient with meningitis caused by this organism.

Among other conditions treated by sulphanilamide, the bacteriology of which is uncertain, are gangrenous balanitis (Fagerstrom, 1937) and acute appendicitis (Dundas, 1937). Maier (1938) cured twelve of fourteen rats of " sniffles " by giving them 50 mgm. of sulphanilamide in their food daily. " Sniffles " in rats is a chronic form of pneumonia, usually fatal. A disease known as infectious paraplegia of horses, apparently a form of glanders, has been found by Velu, Zottner and Miégeville (1937) to be greatly improved by treatment with rubiazol.

Lanier (1938) has used local applications of sulphanilamide in more than 100 cases of tooth extraction, complicated by pyorrhœa, and in 250 cases of chronic periapical infection. Organisms rapidly disappeared from the sockets. Cokkinis (1938 [2]) has obtained success in the treatment of gingivitis and ulcerative stomatitis when sulphanilamide or M. and B. 693 is preceded by a vaccine. Although the ætiology of chronic ulcerative colitis is still uncertain, Bannick *et al.* (1938) believe that in six of nine uncomplicated cases they have obtained improvement if not absolute cure.

Although sulphanilamide has an action on certain fungi *in vitro* (Fourneau *et al.*, 1936), its effect on mycotic infections *in vivo* does not appear to have been tested,

References

Albright, F., Dienes, L., and Sulkowitch, H. W., 1938, Pyelonephritis with nephrocalcinosis caused by *Hæmophilus influenzæ* and alleviated by sulfanilamide : report of two cases. *J. Amer. med. Ass.*, CX., 357.

Bannick, E. G., Brown, A. E., and Foster, F. P., 1938, Therapeutic effectiveness and toxicity of sulphanilamide and several related compounds. *Ibid.*, CXI., 770.

Basman, J., and Perley, A. M., 1937, Report of patients treated with sulfanilamide at St. Louis Children's Hospital. *J. Pediat.*, XI., 212.

Cokkinis, A. J., 1938 [1], Sulphonamide in genito-urinary (non-gonococcal) infections. *Brit. med. J.*, *i.*, 1151.

Cokkinis, A. J., 1938 [2], Sulphonamide chemotherapy in surgical infections. *Brit. med. J.*, *ii.*, 845.

Cruickshank, J. C., 1938, Sulphanilamide and " M and B 693 " in experimental pertussis in mice. *Lancet*, *ii.*, 310.

Dundas, J., 1937, Prontosil in appendicitis. *E. Afr. med. J.*, XIV., 208.

Fagerstrom, D. P., 1937, Sulfanilamide therapy in erosive and gangrenous balanitis. *Urol. cutan. Rev.*, XLI., 858.

Fourneau, E., Tréfouël, J., Tréfouël, Mme., Nitti, F., and Bovet, D., 1936, Action du para-aminophenylsulfamide sur les moisissures. *C. R. Soc. Biol. Paris*, CXXII., 652.

Gross, P., Cooper, F. B., and Lewis, M., 1938, Chemotherapy of *B. pertussis* infection of mice. *Proc. Soc. exp. Biol., N.Y.*, XXXVIII., 407.

Jones, H. W. E., 1937, *H. influenzæ* meningitis. *Brit. med. J.*, *ii.*, 797.

Lanier, W. D., 1938, Sulfanilamide in oral infections. *Med. Bull. Vet. Admin.*, XV., 65.

Long, P. H., and Bliss, E. A., 1937, Para-aminobenzenesulfonamide and its derivatives : experimental and clinical observations on their use in the treatment of beta-hemolytic streptococcic infection. *J. Amer. med. Ass.*, CVIII., 32.

McIntosh, R., Wilcox, D. A., and Wright, H. F., 1937, Results of sulfanilamide treatment at the Babies' Hospital, New York City. *J. Pediat.*, XI., 167.

Maier, N. R. F., 1938, The treatment of " sniffles " in the rat with sulfanilamide. *Science*, LXXXVII., 439.

Povitsky, O. R., 1937, Immune serum and prontosil-combined treatment for the protection of the mouse against fatal dose of *B. influenzæ* meningitis —preliminary report. *N. Y. St. J. Med.*, XXXVII., 1748.

Taylor, H. W., 1938, Treatment of a case of influenzal meningitis with immune serum and sulfanilamide. *Arch. Pediat.*, LV., 131.

Teggart, B., 1938, Influenzal meningitis treated with soluseptasine and lumbar puncture : recovery. *Brit. med. J.*, *i.*, 1365.

Velu, H., Zottner, G., and Miégeville, J., 1937, Les colorants azoiques dans le traitement de la paraplégie infectieuse du cheval. *Bull. Acad. vét. France*, X., 48.

Young, R. H., and Moore, C., 1938, Influenzal meningitis—report of a case treated with anti-influenza serum and sulphanilamide. *Arch. Ped.*, LV., 282.

METHODS OF ADMINISTRATION AND DOSAGE

Prontosil and sulphanilamide have been administered orally, subcutaneously, intramuscularly, intravenously, intrathecally, per rectum, into the pleural cavity and by local application.

Sufficient experience has not yet been gained to allow of definite statements being made as to the best method of administration in different types of infection. Thus in view of the rapidity with which sulphanilamide reaches the central nervous system after oral administration, the question arises whether intrathecal administration is really necessary except in cases where oral administration is out of the question. Schwentker, Gelman and Long (1937) thought that prontosil soluble given intrathecally irritated the meninges, but rubiazol has been given by this route without causing irritation, while Millett (1937), who examined the central nervous system of a fatal case of pneumococcal meningitis treated intrathecally with prontosil soluble, found no sign of injury or irritation from its presence. A 0·8 per cent. solution of sulphanilamide in physiological saline has also been injected intrathecally. If given by this route in any form, sulphonamide compounds must be administered slowly and by gravity. Sulphanilamide may also be given as a 0·8 per cent. solution subcutaneously: amounts up to 700 c.c. have been administered as an initial dose. Uncertainty also still exists as to the value of sulphanilamide injected into the pleural cavity in cases of empyema. In all acute infections it is now realised that sulphonamide drugs must be given in the same manner as digitalis (Marshall, 1938) by rapidly attaining and maintaining a certain concentration of the drug. Thus an initial dose of 3 to 5 gm. is suggested, while, if four hours later the concentration in the blood is below 10 mgm. per 100 c.c., Long and Bliss (1937[1]) recommend subcutaneous injections. Subsequent smaller doses should be given every four hours. The majority of observers now prefer, at any rate in acute infections, to combine sulphanilamide by mouth with intramuscular injections of prontosil soluble. The total daily dosage of sulphanilamide suggested is 1 gm. per 20 lb. of body weight, with a maximum of 5 gm. in a day, accompanied by 0·5 c.c. of prontosil soluble (5 per cent. solution) per lb. of body weight, with a maximum of 60 c.c. When the 2·5 per cent. solution is used these figures, of course, require doubling. In some instances the dose of sulphanilamide has been greater, although 7 gm. daily has rarely been exceeded. In the case of children, Long and Bliss (1937[2]) recommend a 50 per cent. larger dose in proportion to body weight than for adults. With abatement of the acute symptoms the above dosage can be reduced after two or three days, and in more chronic cases smaller doses can be given from the first. The exact length of time for which treatment should be continued is still somewhat uncertain, though it is now generally agreed that, to avoid toxic effects on the hæmatopoietic system, sulphonamide drugs should preferably not be continued for more than ten days on end and certainly not for more than a fortnight. While prontosil is usually given orally, it has been employed for local application to the skin in a saturated solution in equal parts of alcohol and acetone. Prontosil soluble is injected intramuscularly, but given as an intravenous injection it possesses certain dangers. Mixed with varying amounts of glycerine up to 20 per cent., it has been used as a gargle and for painting mucous surfaces.

Sulphanilamide is generally given orally, but a solution containing 0·5 to 0·8 per cent. can be made by grinding the tablets in water or physiological saline, filtering and boiling the filtrate. For local application, the following is recommended : sulphanilamide, 1·5 parts ; alcohol, 90 per cent., and acetone each 7·5 parts, and water to 100 parts (Bosse, 1938). This mixture can be used as a throat spray or vaginal douche. Sulphanilamide ointment, made with lanoline or vaseline as a base, has been employed to a very limited extent.

References

Bosse, G., 1938, Über die Möglichkeit der örtlichen Prontosilanwendung in der inneren Klinik. *Dtsch. med. Wschr.*, IV., 116.

LONG, P. H., and BLISS, E. A., 1937 [1], Use of *para*-aminobenzenesulphonamide (sulphanilamide) or its derivatives in treatment of infections due to beta-hemolytic streptococci, pneumococci and meningococci. *Sth. med. J., Nashville*, XXX., 479.

LONG, P. H., and BLISS, E. A., 1937 [2], Observations upon the experimental and clinical use of sulphanilamide in treatment of certain infections. *Canad. med. Ass. J.*, XXXVII., 457.

MARSHALL, E. K., Jr., 1938, Certain phases of the pharmacologic properties of sulfanilamide. *Med. Ann. District of Colombia*, VII., 5.

MILLETT, J., 1937, Intrathecal use of prontosil soluble: report of case of Type III pneumococcus meningitis and septicæmia treated with prontosil soluble with complete autopsy report. *J. Amer. med. Ass.*, CIX., 2138.

SCHWENTKER, F. F., GELMAN, S., and LONG, P. H., 1937, Treatment of meningococci meningitis with sulfanilamide: preliminary report. *Ibid.*, CVIII., 1407.

TOXIC REACTIONS

As with every other chemotherapeutic remedy a number of toxic reactions have been ascribed to the administration of drugs of the sulphonamide group. The exact incidence of these reactions is at present unknown, since the figures noted by various investigators vary greatly: nor is there any statistical evidence of value to show that reactions are more frequent after one particular compound, a view which has been entertained on the continent of Europe in regard to sulphanilamide as compared with prontosil.

Fortunately most of the reactions described have been mild, and, apart from staining of the tissues with prontosil and prontosil soluble, have consisted of cyanosis, nausea, vomiting, headache, palpitation, dizziness and epistaxis. More serious are the skin reactions with occasional reports of exfoliative dermatitis, and the effects on the hæmatopoietic system associated with agranulocytosis and acute hæmolytic anæmia.

The cause of the majority of these reactions is as yet unknown. Sulphæmoglobinæmia, it is suggested, is due to the ingestion or liberation in the intestine of sulphur. The skin lesions may be due either to allergic manifestations or to sensitisation with porphyrin, which is formed in considerable amounts. Other symptoms suggest a hypersensitivity on the part of certain individuals, and certainly in the genesis of agranulocytosis and hæmolytic anæmia personal idiosyncrasy plays a large part. At present, unfortunately, there is no way of determining which persons are especially liable to react to sulphonamide derivatives. Children, fortunately, appear to be less liable than adults to develop severe reactions.

The tragic occurrence in the United States of America whereby no less than seventy-three persons died after taking sulphanilamide by mouth was due, not to the toxic properties of the drug itself, but to a misguided attempt to produce a stable solution of sulphanilamide for oral administration (*cf.* Geiling and Cannon, 1938). Such a stable solution was obtained by dissolving sulphanilamide in diethylene glycol, the toxicity of which for animals had already been well established (von Oettingen and Jirouch, 1931). As a result of the action of diethylene glycol on the kidneys, death occurred from uræmia in a high proportion of those who had been so unfortunate as to take the drug.

This tragedy emphasises the folly of permitting dangerous compounds to be purchased directly by the consumer without adequate supervision.

The reactions associated with the sulphonamide group of drugs may be provisionally classified as follows :—

(1) **Reactions Affecting the Nervous System.** Mental disturbances, dizziness, palpitation, headache, " drug " fever, sometimes associated with skin rashes, optic neuritis, tinnitus, paræsthesia, peripheral neuritis.

(2) **Reactions Affecting the Skin.** Maculopapular and urticarial rashes, sometimes associated with exposure to light : exfoliative dermatitis.

(3) **Reactions Affecting the Hæmatopoietic System.** Leucopenia, agranulocytosis, acute hæmolytic anæmia, thrombocytopenia.

(4) **Reactions Affecting the Urogenital System.** Acute nephritis : urinary irritation, albuminuria, nitrogen retention, partial azospermia (?).

(5) **Reactions Affecting the Digestive System.** Nausea, vomiting, diarrhœa, constipation, toxic hepatitis (?).

(6) **Reactions Affecting Pigments.** (*a*) Cyanosis ; (*b*) methæmoglobinæmia ; (*c*) sulphæmoglobinæmia ; (*d*) excessive porphyrin formation.

As an example of the relative frequency of these different reactions, the figures obtained by Hageman and Blake (1938) in the treatment of 114 patients with beta-hæmolytic streptococcal infections may be taken as typical.

COMPLICATIONS ASSOCIATED WITH SULPHANILAMIDE TREATMENT OF 114 PATIENTS (Hageman and Blake, 1938)

Complication.	No. of cases.	Percentage.
Cyanosis	51	44·7
Drug fever	17	14·9
,, ,, (with rash)	9	7·9
,, ,, (without rash)	8	2·6
Nitrogen retention	3	0·9
Hepatitis	1	0·9
Secondary anæmia	1	0·9
Thrombocytopenia	1	0·9
Total number of patients with complications excluding cyanosis	22	19·3
Total number of patients with all complications	56	49·1

References

GEILING, E. M. K., and CANNON, P. R., 1938, Pathologic effects of elixir of sulfanilamide (diethylene glycol) poisoning: a clinical and experimental correlation: final report. *J. Amer. med. Ass.*, **CXI.**, 919.

HAGEMAN, P. O., and BLAKE, F. G., 1938, Clinical experience with sulphanilamide in the treatment of beta-hemolytic streptococcic infection. *Amer. J. med. Sci.*, **CXCV.**, 163.

VON OETTINGEN, W. F., and JIROUCH, E. A., 1931, The pharmacology of ethylene glycol and some of its derivatives in relation to their chemical constitution and physical chemical properties. *J. Pharm.*, **XLII.**, 355.

MENTAL AND NERVOUS DISTURBANCES

In view of the effect of sulphanilamide and allied compounds on the central nervous system of animals, previously described, it is perhaps surprising that so few cases of nervous involvement have been reported in man. Headache, chiefly frontal, lassitude, anorexia, dizziness, drowsiness, mental depression, disorientation, somnolence, loss of memory, paræsthesia and loss of taste are, however, not uncommon, while actual mental disturbance, tingling in hands and feet, tinnitus and a slight degree of deafness have also been recorded. Schreus (1938) has reported a motor peripheral neuritis after 36·5 gm. of sulphanilamide. Hogan and McNamara (1938) believe that psychosis was, in one instance, precipitated by the administration of sulphanilamide, while Wyman (1938) has recorded persistent hiccough, probably central in origin. Several abdominal cramps have also been reported.

The most alarming nervous disturbance, however, is that recorded by Bucy (1937), who treated a girl aged sixteen with sulphanilamide for chronic osteomyelitis. Defective vision appeared, and on examination absolute scotomata for red and blue and relative scotomata for white were discovered, while the discs were slightly swollen and reddened. After suspending treatment it took about a month for normal vision to be restored.

The administration of uleron and DB 87 has been associated with a peculiar form of peripheral motor neuritis, of symmetrical distribution, affecting more especially the flexor muscles of the wrists, the interosseous, adductor and opponens pollicis muscles of the hands, the flexors of the ankle joints, the external pterygoids. The paralysis is often of long duration (*cf.* Tietze, 1938 ; Lemke, 1938 ; Hullstrung and Krause, 1938, and others). Bürger (1938) notes a case of what he terms polyneuromyositis in a thirty-one-year-old male with gonorrhœa. Sciatica may also occur, while paræsthesia, dysæsthesia and disturbance of the autonomic system have been noted.

References

Bucy, P. C., 1937, Toxic optic neuritis resulting from sulfanilamide. *J. Amer. med. Ass.*, CIX., 1007.

Bürger, L., 1938, Polyneuromyositis nach Ulironbehandlung schwerer Gonorrhöe. *Dtsch. med. Wschr.*, LXIV., 709.

Freusberg, O., 1938, Polyneuritis nach Ulironverabreichung. *Ibid.*, LXIV., 776.

Hogan, B. W., and McNamara, P. J., 1938, Psychosis precipitated by sulfanilamide. *Nav. med. Bull., Wash.*, XXXVI., 60.

Hullstrung, H., and Krause, F., 1938, Polyneuritis nach sulfonamidhaltigen Verbindungen bei Menschen und Tauben. *Dtsch. med. Wschr.*, LXIV., 114.

Lemke, R., 1938, Über Neuritis nach Uliron. *Münch. med. Wschr.*, LXXXV., 452.

Schreus, H. T., 1938, Praxis der Uliron-Behandlung der Gonorrhöe. *Med Welt.*, XII., 11.

TIETZE, A., 1938, Periphere Lähmungen nach Ulironbehandlung. *Münch. med. Wschr.*, LXXXV., 332.
WYMAN, M. H., 1938, Unusual reaction from sulfanilamide. *South. med. J.*, C., 15.

DRUG FEVER

Attention was called by Colebrook *et al.* (1937) to the fact that in 29·3 per cent. of non-fatal cases of puerperal fever treated with sulphanilamide, a secondary rise of temperature occurs usually seven to ten days after the onset of the drug treatment and after the temperature has fallen to normal. The secondary rise often occurs while the sulphanilamide is actually being given and falls as soon as administration ceases. Hageman and Blake (1937) have described similar cases in the United States. This drug fever must be distinguished both from a recrudescence of the bacterial infection and from fever associated with drug eruptions. In certain instances the action of the drug on the bacteria appears to liberate toxin, causing an acute reaction not unlike a malarial attack.

References

BARBER, H. W., 1938, Facial carbuncle treated with prontosil album. *Lancet, ii.*, 668.
COLEBROOK, L., and PURDIE, A. W., 1937, Treatment of 106 cases of puerperal fever by sulphanilamide (streptocide). *Lancet, ii.*, 1237 and 1291.
HAGEMAN, P. O., and BLAKE, F. G., 1937, Specific febrile reaction to sulfanilamide : drug fever. *J. Amer. med. Ass.*, CIX., 642.

SKIN LESIONS

In approximately 6 per cent. of cases (*cf.* Schwentker and Gelman, 1937, 10 cases in 180 patients) skin rashes have been described as a result of the use of drugs of the sulphonamide series, more especially sulphanilamide. Patients with rheumatism are especially liable, for of fifty-eight no less than thirty-one developed rashes (Massel and Jones, 1938). The rash may appear as early as the third, or as late as the fourteenth to twentieth day of treatment. The majority of rashes appear about the eighth or ninth day and are described as either purpuric (Goodman and Levy, 1937), scarlatiniform, morbilliform or maculopapular. The area affected varies considerably : in some patients the rash is confined to the buttocks, palms of the hands and soles of the feet ; in others exposed skin surfaces, such as the face, neck and back of the hands are alone affected, or the rash may involve the whole of the trunk. Mucous membranes are not affected. According to Menville and Archinard (1937), severe itching often accompanies the skin lesions, which may be associated with fever, œdema, albuminuria and icterus. A number of observers (Finney, 1937 ; Myers *et al.*, 1937 ; Saphirstein, 1938) have described symptoms resembling the exfoliative dermatitis met with after the use of arsphenamine compounds. As a rule the skin symptoms clear up rapidly when the administration of the drug ceases. Saphirstein (1938), however, described a case in which desquamation of the skin came on seven days after the drug had been stopped. The renewed use of sulphonamide as a rule causes a return of the skin lesions (Unshelm, 1937 ; Goodman and Levy, 1937 ; Newman and Sharlit, 1937). This fact, combined with the somewhat capricious incidence of the condition and the small amount of the drug necessary to produce it, has led to the suggestion of an individual idiosyncrasy on the part of affected patients to the drug employed (Schonberg, 1937). Goodman and Levy (1937), however, were unable to detect any evidence of allergy, though Salvin (1937) obtained positive " patch " tests in one patient. Massel and Jones (1938) found that in nine patients showing skin reactions the continued administration of small doses eventually produced a state of tolerance.

Since the skin lesions often appear on exposed areas it has been suggested that photosensitisation may play a part in the production of sulphanilamide rashes, and Brunsting (1937) in fact found that in two patients with dermatitis the urinary porphyrin was increased. The question of porphyrinuria following sulphanilamide has been further investigated by Rimington and Hemmings (1938), who found that the urine of patients treated with sulphanilamide contained abnormally large amounts of ether-soluble porphyrins. The administration to normal rats per mouth of doses of from

0·079 to 0·307 gm. per day for periods up to thirty days showed increases in urinary and fæcal porphyrin excretion up to ten times the normal, although no other signs of toxicity were seen. The presence of a slight degree of photosensitivity in rats was determined as a result of exposure to ultra-violet light. Newman and Sharlit (1937) noted photosensitivity in four patients after treatment with sulphanilamide. In rats, coproporphyrin III, with a trace of coproporphyrin I, were found in the urine, and the same two pigments with a greater proportion of series I isomer in the fæces. From human urines coproporphyrins I and III were isolated in approximately equal quantity. The series III pigments are less active as photosensitisers than their series I analogues, but it is possible that certain individuals may produce more of series I than others. In this connection it is of interest to note that the symptoms exhibited by patients suffering from acute porphyrinuria, nausea, colic, vomiting, constipation and muscular weakness, bear a certain resemblance to those produced by sulphanilamide.

Rimington and Hemmings (1938) showed that rats killed at the height of sulphanilamide dosing show a deposit in the spleen of greenish-brown granular pigment, much stainable iron and a certain amount of porphyrin. Although sulphanilamide undoubtedly causes some degree of red-cell destruction, it probably has a more deep-seated effect upon the hæmopoietic system. It is tempting to compare the situation with that in lead poisoning, where (*cf.* Rimington, 1938) the drug probably acts by interfering with the mechanism of the incorporation of iron into protoporphyrin to produce hæmatin.

Skin rashes have also been reported after treatment by uleron. In one fatal case, described by Euler (1938), in addition to lesions resembling exfoliative dermatitis, the number of leucocytes was reduced to 3,800 per c.m. and ulcerative lesions were present on the epiglottis.

References

Brunsting, L. A., 1937, Sulfanilamide dermatitis: question of relation to photosensitivity. *Proc. Staff Meet. Mayo Clinic*, XII., 614.

Euler, H. E., 1938, Zur Ulironfrage. *Münch. med. Wschr.*, LXXXV., 623.

FINNEY, J. O., 1937, Severe dermatitis medicamentosa following the administration of sulfanilamide. *J. Amer. med. Ass.*, CIX., 1982.

GOODMAN, M. H., and LEVY, C. S., 1937, The development of a cutaneous eruption (toxicodermatosis) during the administration of sulfanilamide. *Ibid.*, CIX., 1009.

MASSEL, B. F., and JONES, T. D., 1938, The effect of sulfanilamide on rheumatic fever and chorea. *New. Eng. J. Med.*, CCXVIII., 876.

MENVILLE, J. G., and ARCHINARD, J. J., 1937, Skin eruptions in patients receiving sulfanilamide : report of four cases. *J. Amer. med. Ass.*, CIX., 1008.

MYERS, G. B., VONDER HEIDE, E. C., and BALCERSKI, M., 1937, Exfoliative dermatitis following sulphanilamide. *Ibid.*, CIX., 1983.

NEWMAN, B. A., and SHARLIT, H., 1937, Sulfanilamide : a photosensitising agent of the skin. *Ibid.*, CIX., 1036.

RIMINGTON, C., 1938, An enzymic theory of hæmopoiesis. *C. R. Lab. Carlsberg*, XXII., 454.

RIMINGTON, C., and HEMMINGS, A. W., 1938, Porphyrinuria following sulphanilamide : sulphanilamide dermatitis. *Lancet*, *i.*, 770.

SALVIN, M., 1937, Hypersensitivity to sulfanilamide. *J. Amer. med. Ass.*, CIX., 1038.

SAPHIRSTEIN, H., 1938, Hepatitis and toxic erythema with desquamation due to sulfanilamide. *Urol. cutan. Rev.*, CCXVI., 101.

SCHONBERG, I. L., 1937, Purpuric and scarlatiniform eruption following sulfanilamide. *J. Amer. med. Ass.*, CIX., 1035.

SCHWENTKER, F. F., and GELMAN, S., 1937, Sulfanilamide rash. *Johns Hopk. Hosp. Bull.*, LXI., 75.

UNSHELM, E., 1937, Zur Behandlung von Kindern mit Prontosil. *Arch. Kinderh.*, CX., 76.

HYPERSENSITIVITY

An interesting case of hypersensitivity to sulphanilamide is recorded by Salvin (1937), who reported itching, sneezing, shortness of breath and lachrymation after the administration of sulphanilamide. Patch tests with sulphanilamide gave positive results and the symptoms could be produced with regularity by giving small doses of the drug. The patient, whose case was reported by Bucy (1937) as one of optic neuritis, showed intolerance to sulphanilamide, since each administration of the drug was followed by headache, diarrhœa and a choking sensation. Hageman and Blake (1937) observed in nine patients fever, malaise, nausea, itching, tinnitus and maculopapular rashes, occasionally accompanied by weals and hæmorrhagic lesions. The skin rashes affected not only the exposed parts, but the buttocks, knees and back as well. These symptoms were regarded as probably allergic in character although they obtained no evidence of sensitisation to sulphanilamide.

References

Bucy, P. C., 1937, Toxic optic neuritis resulting from sulfanilamide. *J. Amer. med. Ass.*, CIX., 1007.

Hageman, P. O., and Blake, F. G., 1937, Specific febrile reaction to sulfanilamide : drug fever. *Ibid.*, CIX., 642.

Salvin, M., 1937, Hypersensitivity to sulfanilamide. *Ibid.*, CIX., 1038.

THE EFFECT ON THE HÆMATOPOIETIC SYSTEM

In view of the reported occurrence of leucopenia and agranulocytosis, the effect of sulphanilamide and prontosil on the hæmatopoietic system is of considerable importance.

In rabbits, Finklestone-Sayliss, Paine and Patrick (1937) found that the reticulocyte count remained almost constant despite doses as high as 1·2 gm. of sulphanilamide per day. Abnormal and nucleated red blood corpuscles were, however, seen. Marshall, Cutting and Emerson (1938) found no effect on the blood picture of dogs and rabbits after the administration of large doses, 4 to 6 gm. per kilogram of body weight in four or five days, of sulphanilamide.

In man, Campbell (1938), in a series of ten cases, noted a slight rise in the number of reticulocytes, not, however, associated with anæmia or leucopenia. He concluded, therefore, that moderate doses of sulphanilamide act as a mild stimulus to the bone marrow. Bigler *et al.* (1938) studied the leucocyte response to sulphanilamide in thirty-three patients. The hæmoglobin percentage, the number of red blood corpuscles and the number of blood platelets were unaffected. The proportion of polymorphonuclear leucocytes was not increased but the leucocyte count fell, on recovery, in all but three patients, and in two there was actual leucopenia. Although no agranulocytosis was seen, it is suggested that sulphanilamide depresses the white cells. On the other hand, Osgood and Brownlee (1938) found that a concentration of sulphanilamide 1 in 1,000 had no effect on cultures of cells from human bone marrow over a period of eight days.

The most extensive investigations have been made by Britton and Howkins (1938), who made serial leucocyte counts on fifty ambulant patients receiving 21 gm. of sulphanilamide in fourteen days. In twenty-three a transient polymorphonuclear leucopenia and in twenty-two a monocytosis occurred. These changes were usually found between the seventh and twentieth days after beginning the drug. Toxic reactions bore no definite relationship to the leucocyte changes, but in some instances, though not invariably, eosinophilia was associated with skin rashes.

When prontosil was given to rabbits in doses of 0·25 gm. per kilogram of body weight, Kreutzman and Carr (1938) found no numerical change in the cellular elements of the blood in twenty-one days. An increase, however, in reticulocytes and in eosinophilic polymorphonuclear leucocytes occurred, suggesting a mild depression of the bone, a suggestion strengthened by the congestion of the spleen and the increase of eosinophils in the bone marrow. Domagk (1935), giving 0·1 to 0·2 gm. orally to rabbits for fourteen days, observed no change in the blood picture.

Observations on the effect of prontosil soluble on the blood and bone marrow have not been reported.

References

Bigler, J. A., Clifton, W. M., and Werner, M., 1938, The leukocyte response to sulfanilamide therapy. *J. Amer. med. Ass.*, CX., 343.

Britton, C. J. C., and Howkins, J., 1938, Action of sulphanilamide on leucocytes : a report on fifty ambulant patients. *Lancet, ii.*, 718.

Campbell, C. M., Jr., 1938, Effect of sulphanilamide on the blood picture. *Ibid.*, *i.*, 247.

Domagk, G., 1935, Ein Beitrag zur Chemotherapie der bakteriellen Infektionen. *Dtsch. med. Wschr.*, LXI., 250.

Finklestone-Sayliss, H., Paine, C. G., and Patrick, L. B., 1937, Bacteriostatic action of *p*-aminobenzene-sulphonamide upon hæmolytic streptococci. *Lancet, ii.*, 792.

Kreutzman, W., and Carr, J. L., 1938, Effect of prontosil on blood cells. *Proc. Soc. exp. Biol., N.Y.*, XXXVIII., 19.

Marshall, E. K., Jr., Cutting, W. C., and Emerson, E. K., Jr., 1938, Toxicity of sulfanilamide. *J. Amer. med. Ass.*, CX., 252.

Osgood, E. E., and Brownlee, I. E., 1938, Mode of action of sulfanilamide. *Ibid.*, CX., 349.

AGRANULOCYTOSIS

Although fortunately rare, agranulocytosis is the most serious complication associated with the administration of sulphonamide and prontosil, since it has caused a number of deaths. The exact rôle played by the drugs in producing this condition is somewhat difficult to assess, since the number of cases of agranulocytosis which has occurred is extraordinarily small in proportion to the vast number of persons who have received these drugs.

Up to the present fifteen cases of agranulocytosis have been reported, six of which recovered. The fatal cases are as follows :—

(1) A woman of sixty-one who had suffered for ten years from thrombopenic purpura : as a result of pyelocystitis she received 79 gm. of prontosil in five weeks. Signs of pneumonia and fever developed, with difficulty in swallowing and almost complete disappearance of polymorphonuclear leucocytes (Borst, 1937).

(2) A man of fifty-three with acute rheumatism received 54 gm. of sulphanilamide in eighteen days. Four days after the end of treatment complete agranulocytosis developed (Young, 1937).

(3) A man, aged twenty, suffering from acute rheumatism and possibly bacterial endocarditis, was given 54 gm. of *p*-benzylaminobenzenesulphonamide (proseptasine) in eighteen days : two days later pharyngeal ulceration appeared, the leucocytes fell to 300 per c.c. and death occurred (Model, 1937).

(4) A woman, aged fifty-four, suffering from subacute bacterial endocarditis, died of agranulocytosis after receiving 47 gm. in roughly six weeks (Plumer, 1937).

(5) A thirty-two-year-old man with a chronic penile ulcer received 56·6 gm. of sulphanilamide in twenty-one days : leucopenia was followed by agranulocytic angina (Schwartz *et al.*, 1938).

(6) A man, aged twenty-two, with acute gonorrhœa, was given 38 gm. of sulphanilamide in short courses with rest intervals (Berg and Holtzman, 1938).

(7) A man, aged sixty-three, suffering from pyelitis, was treated first with prontosil and later with sulphanilamide. On the eighteenth day of treatment he developed agranulocytosis and died (Gompertz, Groen and de Vries, 1937).

(8) A man, aged twenty-two, suffering from gonorrhœa, was treated with sulphanilamide, receiving in seven days 23·6 gm.

His white blood cells fell first to 3,500 and later to 650 per c.mm. shortly before death (O'Connell, 1938).

(9) A woman, aged twenty-two, suffered from a mild pre-eclamptic toxæmia : on the second day after delivery she developed a local uterine infection, and over a period of seventeen days was given 78·5 gm. of prontosil and sulphanilamide. On the twenty-fourth day the white cells fell to 1,500 per c.mm. and next day to 450 per c.mm. with few polymorphonuclear leucocytes. Death took place on the thirtieth day (Brown, 1938).

In six other cases recovery has occurred.

(10) A woman, aged thirty-five, suffered from rheumatoid arthritis and received 28·8 gm. of sulphanilamide in thirty-two days when the white cells fell to 450 per c.mm. with no granular cells. Pentonucleotide produced a cure (McGuire and McGuire, 1938).

(11) A woman received 94·5 gm. of sulphanilamide in three weeks. The white blood count fell to 2,000 per c.mm., with 2 per cent. of polymorphonuclear leucocytes, and later to 444 per c.mm. : treatment with pentnucleotide and campolon was followed by recovery (Jennings and Southwell-Sander, 1937).

(12) A man, aged nineteen, suffering from syphilis and gonorrhœa, received 47·5 gm. of sulphanilamide in seventeen days in addition to four injections of bivatol and two of sulpharsphenamine. Recovery followed blood transfusions and activated vegetable charcoal administration (Touraine *et al.*, 1937).

(13) A parturient woman received 60 gm. of sulphanilamide in three weeks : acute anæmia ensued and a fall in the polymorphonuclear count to 12 per cent. Sulphanilamide was discontinued and campolon and iron given (Bayer Products, 1938).

(14) A girl of fourteen years of age was operated on for a hydronephrosis which had ruptured after trauma. Three weeks after operation the patient became febrile and the urine contained bacteria and polymorphonuclear leucocytes. For twenty-five days 0·9 gm. of prontosil was given daily : at the end of this time the leucocytes fell to 5,800 per c.mm. with an entire absence of polymorphonuclear leucocytes. The red cells were 2,800,000 per c.mm. With the aid of blood transfusions and iron recovery eventually took place (Soedjono, 1937).

(15) A girl of eighteen, with an acute Bartholin's abscess, was

given 18 gm. of sulphanilamide in nineteen days. The white cell count fell from 10,500 to 2,400 per c.mm. with a complete absence of polymorphonuclear leucocytes (Allen and Short, 1938).

In all these patients the dosage was fairly high and treatment had been carried out for some weeks before the symptoms of agranulocytosis appeared. In addition to the fatal cases, two cases of severe leucopenia, affecting the polymorphonuclear leucocytes, have been recorded by Trumper (1937) after treatment with prontosil and sulphanilamide. After withdrawal of the drugs the leucocyte count increased. On the other hand, sulphanilamide has been successfully used in the treatment of two cases of agranulocytosis. Fitz-Hugh (1937) reported the case of a patient with agranulocytic angina following amidopyrine treatment and a secondary hæmolytic streptococcal infection. A similar case was published by Krauel (1937). A condition closely resembling agranulocytosis has been described by Rotter (1937) in a patient with gonorrhœa treated with uleron.

References

ALLEN, J. G., and SHORT, C. L., 1938, Granulocytopenia associated with sulfanilamide therapy. *New Eng. med. J.*, CCXIX., 6.

BAYER PRODUCTS LTD., 1938, "Prontosil—a survey of the new chemotherapy." London.

BERG, S., and HOLTZMAN, M., 1938, Fatal granulocytopenia following sulfanilamide therapy. *J. Amer. med. Ass.*, CX., 370.

BORST, J. G. G., 1937, Death from agranulocytosis after treatment with prontosil flavum. *Lancet*, *i.*, 1519.

BROWN, D. B., 1938, Treatment of puerperal sepsis by prontosil and allied compounds. *J. Obst. Gynæc. Brit. Emp.*, XLV., 495.

FITZ-HUGH, T., Jr., 1937, The etiology and pathology of agranulocytic angina. *Amer. J. clin. Path.*, VII., 524.

GOMPERTZ, J. J., GROEN, J., and DE VRIES, S. I., 1937, Agranulocytose na gebruik van prontosil. *Ned. Tijdschr. Geneesk.*, LXXXI., 5932.

JENNINGS, G. H., and SOUTHWELL-SANDER, G., 1937, Anæmia and agranulocytosis during sulfanilamide therapy. *Lancet*, *ii.*, 898.

KRAUEL, G., 1937, Beitrag zur experimentellen Agranulocytose. *Klin. Wschr.*, XXXVI., 1245.

McGUIRE, P. R., and McGUIRE, J. P., 1938, Agranulocytosis following use of sulphanilamide: report of case. *Illinois med. J.*, LXXIII., 425.

MODEL, A., 1937, Agranulocytosis and para-aminobenzenesulphonamide. *Brit. med. J.*, *ii.*, 295.

O'CONNELL, J. T., 1938, Sulfanilamide poisoning: report of a fatal case. *Nav. med. Bull., Wash.*, XXXVI., 61.

PLUMER, H. E. 1937, Prontylin and prontosil. *New Engl. J. med.*, CCXVI., 711.

Rotter, H., 1937, Akute Exantheme im Verlauf moderner Gonorrhöebehandlung. *Wien. klin. Wschr.*, **L.**, 1744.

Schwartz, W. F., Garvin, C. F., and Koletsky, S., 1938, Fatal granulocytopenia from sulfanilamide. *J. Amer. med. Ass.*, **CX.**, 368.

Soedjono, D. P., 1937, Agranulocytose door Prontosil. *Maandschr. Kindergeneesk.*, **VII.**, 82.

Touraine, A., Durel, P., and Baudouin, A., 1937, Agranulocytose après para-aminophénylsulfamide au cours d'un traitement anti-blennorragique. *Bull. Soc. franç. Dermat. Syph.*, **XLIV.**, 2072.

Trumper, A., 1937, Prontylin and prontosil. *New. Engl. J. med.*, **CCXVI.**, 857.

Young, C. J., 1937, Agranulocytosis and para-aminobenzenesulphonamide *Brit. med. J.*, *ii.*, 105.

ANÆMIA

Unlike agranulocytosis, which develops after some weeks' treatment, an occurrence which affords a strong argument for limiting the administration of sulphanilamide to a period of not more than a fortnight, acute hæmolytic anæmia occurs much earlier, usually between the third and seventh days.

Fortunately this form of anæmia is quite as rare as agranulocytosis and less than twenty cases have so far been reported. As in the cases of agranulocytosis, personal idiosyncrasy appears to play a large part in the genesis of the condition, while in some instances the bacterial infection itself is probably responsible. In three cases reported by Harvey and Janeway (1937), and in the single case recorded by Kohn (1937), the anæmia developed suddenly, once on the second, twice on the fifth and once on the seventh day after treatment had been begun. In addition to extreme pallor and a varying degree of jaundice, the temperature rose, but the hæmoglobin and the number of red cells fell rapidly. Leucocytosis was present and in one patient hæmoglobin appeared in the urine. These four patients all recovered when sulphanilamide was stopped, while two of them were subsequently given small doses of the drug without causing any reappearance of the symptoms. In a case described by Townsend and Mulcahy (1938), the acute anæmia developed after the administration of 9·3 gm. of sulphanilamide given in three days. The icteric index rose to 24·9 and epistaxis was severe. Long and Bliss (1937) recorded seven further cases of hæmolytic jaundice. In a case reported from China by Stannard (1938) the anæmia developed very gradually. In this connection may be mentioned the fact that Singer (1938) has reported a case of hæmoglobinuria occurring in a man who had received 8 gm. of prontosil in four days. Three days after the cessation of treatment the hæmoglobinuria ceased. Longcope (1938) reported a case of hæmolytic anæmia following sulphanilamide therapy in a one-month-old child with streptococcal peritonitis. Greenthal (1938), however, successfully treated a case of streptococcal sepsis in which acute hæmolytic anæmia had developed before the administration of sulphanilamide.

Anæmia, unassociated with hæmolysis but with a faint trace of icterus, was recorded by Weinberg, Mellon and Shinn (1937), while in the cases reported by Jennings and Southwell-Sander (1937) there was neither hæmolysis nor icterus. The case reported by the former authors was one of streptococcal meningitis and the patient was treated by sulphanilamide and prontosil. On the ninth day of treatment there was a moderate decrease in the number of red blood corpuscles and in the amount of hæmoglobin, polychromasia and a moderate degree of anisocytosis, with a faint yellow tint of the skin. With discontinuance of the drug the condition of the blood improved. The three cases of Jennings and Southwell-Sander (1937) were similar and appeared after fairly large doses of the drug. No rise of temperature occurred and anæmia appeared some time after the cessation of treatment. The patients, who were in poor physical condition when treatment was begun, all recovered.

References

GREENTHAL, R. M., 1938, Acute hemolytic anæmia developing during streptococcic sepsis: report of case in which sulfanilamide was given after anæmia had developed. *J. Ped.*, XII., 517.

HARVEY, A. M., and JANEWAY, C. A., 1937, The development of acute hemolytic anemia during the administration of sulfanilamide. *J. Amer. med. Ass.*, CIX., 12.

JENNINGS, G. H., and SOUTHWELL-SANDER, G., 1937, Anæmia and agranulocytosis during sulfanilamide therapy. *Lancet, ii.*, 899.

KOHN, S. E., 1937, Acute hemolytic anemia during treatment with sulfanilamide. *J. Amer. med. Ass.*, CIX., 1005.

LONG, P. H., and BLISS, E. A., 1937, Clinical use of sulphanilamide and its derivatives in treatment of infectious diseases. *Ann. intern. Med.*, XI., 575.

LONGCOPE, W. T., 1938, Problems relating to the invasive properties of hemolytic streptococci and their control by sulphanilamide. *Amer. J. med. Sci.*, CXCV., 577.

SINGER, K., 1938, Hämoglobinurie nach peroraler Prontosiltherapie. *Wien. med. Wschr.*, LXXXVIII., 22.

STANNARD, R. E., 1938, Sulfanilamide : a résumé of available literature with notes on a case of slowly progressing hemolytic anemia. *China med. J.*, LIII., 233.

TOWNSEND, T. M., and MULCAHY, T. M., 1938, Sulfanilamide in the treatment of urological infections. *New York State J. Med.*, XXXVIII., 833.

WEINBERG, M. H., MELLON, R. R., and SHINN, L. E., 1937, Two cases of streptococcic meningitis treated successfully with sulfanilamide and prontosil. *J. Amer. med. Ass.*, CVIII., 1948.

THROMBOCYTOPENIA

As cases have been reported in which both the white and red cells have suffered as a result of the action of sulphanilamide, it is perhaps remarkable that so few instances have been found of involvement of the blood platelets. Hageman and Blake (1938), however, record the history of a man who developed thrombocytopenic purpura. He had been taking sulphanilamide, salol and phenacetin in small doses while being treated for pharyngitis and cervical adenitis. The patient gradually recovered, and, although the ætiology is not quite clear, it seems likely that sulphanilamide may have played some part in the production of the blood dyscrasia.

Reference

HAGEMAN, P. O., and BLAKE, F. G., 1938, Clinical experience with sulphanilamide in the treatment of beta-hemolytic streptococcic infection. *Amer. J. med. Sci.*, CXCV., 163.

ACIDOSIS

A fall in the carbon dioxide-combining power of the plasma was observed by Southworth (1937) in patients both with and without clinical signs of acidosis. In some cases the liver function was depressed and, in addition, there were cyanosis and fever. Long and Bliss (1937) believed that acidosis was associated with the loss of sodium and potassium in the urine and recommended the administration of sodium bicarbonate. Strauss and Southworth

(1938) actually found that in man sulphanilamide caused such a loss of sodium and potassium ions, together with a diuresis. Basman and Perley (1937) recommended 10 c.c. of a molar solution of sodium *r*-lactate in six doses during the twenty-four hours. Since they also observed a decreased carbon dioxide-combining power of the plasma with alkaline urine they believe the condition to be an alkalosis due to hyperpnœa.

Some degree of acidosis appears, at any rate in dogs, to be a necessary accompaniment of sulphanilamide treatment, and is explained by Marshall, Cutting and Emerson (1938) as due to the re-absorption, in the tubules of the kidney, of 70 to 80 per cent. of the drug in the glomerular filtrate whereby, they suggest, there is impairment with re-absorption of bicarbonate and base. This explanation (*cf.* p. 335) is, however, difficult to reconcile with the fact that the *p*H of the serum either remains constant or even rises (Hartmann, Perley and Barnett, 1938).

References

Basman, J., and Perley, A. M., 1937, Report of patients treated with sulfanilamide at St. Louis Children's Hospital. *J. Pediat.*, XI., 212.

Hartmann, A. F., Perley, A. C., and Barnett, H. J., 1938, A study of some of the physiological effects of sulfanilamide. I. Changes in the acid base balance. *J. clin. Invest.*, XVII., 465.

Long, P. H., and Bliss, E. A., 1937, Use of para-aminobenzenesulphonamide (sulphanilamide) or its derivatives in treatment of infections due to beta-hemolytic streptococci, pneumococci and meningococci. *Sth. med. J.*, XXX., 479.

Marshall, E. K., Jr., Cutting, W. C., and Emerson, E. K., 1938, The toxicity of sulfanilamide. *J. Amer. med. Ass.*, CX., 252.

Southworth, H., 1937, Acidosis associated with administration of para-aminobenzenesulfonamide (prontylin). *Proc. Soc. exp. Biol., N.Y.*, XXXVI., 58.

Strauss, M. B., and Southworth, H., 1938, Urinary changes due to sulfanilamide administration. *Bull. Johns Hopk. Hosp.*, LXIII., 41.

CYANOSIS

Very shortly after the introduction of prontosil and sulphanilamide, reports began to appear of cyanosis as a not uncommon result of the administration of these compounds.

Apart from cyanosis due to the bacterial infection itself, and in pneumonia and streptococcal septicæmias such a condition is by no means rare, it is now recognised that there are three possible causes of cyanosis :—

(1) Methæmoglobinæmia.
(2) Sulphæmoglobinæmia.
(3) Unexplained causes.

In the last form, described by Cokkinis (1937), the patient's face, forehead and neck become suffused a rich pink colour. Sprectroscopic examination of the blood does not show any abnormality. Colebrook and Purdie (1937) also failed to detect abnormal absorption bands in the blood of eight cyanosed patients. Marshall and Walzl (1937) have brought forward evidence to show that the oxygen capacity of the blood is not appreciably lowered by cyanosis. On the other hand, Mull and Smith (1938) reported a lowering of the oxygen capacity of the blood in addition to cyanosis. This, however, is denied by Chesley (1938), who found that in eight patients the theoretical CO capacity, calculated from the iron present in the blood, and the measured CO capacity agreed closely. In other words, all hæmoglobin is present in an active form. It is now recognised that as many patients with intense cyanosis have neither methæmoglobin nor sulphæmoglobin present in the blood, the rôle of these pigments in the genesis of the cyanosis appears questionable. Ottenberg and Fox (1938) have found that when dilute solutions of sulphanilamide are exposed to ultra -violet light for a few minutes they develop a strong violet colour which later changes to pinkish purple. When red cells are added to such solution they take up the colour from the solution and assume the colour shown by the red cells of patients under sulphanilamide therapy. Irradiated solutions tested by Marshall's method are found to have lost 30 to 60 per cent. of their sulphanilamide content. It is suggested that coloured derivatives of sulphanilamide may be formed in the tissues.

Although met- and sulphæmoglobinæmia may on occasion occur together, they most commonly appear separately. Sulphæmoglobinæmia, recognised by Colebrook and Kenny (1936), was at first thought to be a rather rare complication, and on the continent of Europe reports of its occurrence are still infrequent. In Great Britain, however, Discombe (1937) has found that some degree of it is the rule during sulphanilamide treatment. This, it has been suggested, is due to the greater consumption of bacon and eggs and other sulphur-containing foods in England when compared with continental countries. Sulphæmoglobinæmia is said to have been absent in 215 cases treated with *p*-benzylaminobenzenesulphonamide (Peters and Havard, 1937). Paton and Eaton (1937) have shown that whereas methæmoglobin disappears within approximately twenty-four hours of the cessation of treatment, sulphæmoglobinæmia may persist for as long as six weeks after administration has ended.

Archer and Discombe (1937) conclude that any drug containing the group C_6H_5N— is capable of giving rise to methæmoglobinæmia. When compounds such as aniline, acetanilide or sulphanilamide enter the blood the amino-group, according to Gley (1937), is oxidised to hydroxylamine. When no sulphur is present in the blood the hydroxylamine is reduced by hæmoglobin which is converted into methæmoglobin. In the presence of sulphur, sulphæmoglobin is formed. This theory requires further investigation. Archer and Discombe (1937), while believing that hydrogen sulphide is normally excreted by the lungs, suggest that an excessive formation of hydrogen sulphide or the presence of a suitable catalyst, a rôle possibly played by sulphanilamide, causes the sulphide to combine with hæmoglobin, with the formation of sulphæmoglobin. To avoid an excess of sulphide all foods containing sulphur must be excluded, a low residue diet should be administered and all purgatives, with the exception of liquid paraffin, must be barred for at least three days before the administration of sulphanilamide.

Pentothal sodium, an anæsthetic given intravenously, also contains sulphur and should not be given in association with sulphanilamide (Hewer, 1938), while, in addition, phenacetin and coal tar drugs must be avoided. Despite these precautions cases of sulphæmoglobinæmia and methæmoglobinæmia continue to occur, possibly in some instances as a result of personal idiosyncrasy to the drug.

The following spectroscopic method, described by Colebrook and

Purdie (1937), is of value, particularly in the detection of cases with abnormal pigments in which cyanosis is not clinically detectable.

Oxalated blood is first laked by the addition of one volume of saponin solution, placed in a test tube of $\frac{3}{8}$ in. diameter and examined with a direct-vision spectroscope. If a band in the red is observed, the blood is further diluted till this can be clearly seen and its approximate wave-length determined. A band between 6,150 and 6,300 A is taken as due to sulphæmoglobin and one between 6,300 and 6,400 A as due to methæmoglobin. As a confirmatory test a drop of very dilute ammonium sulphide is added. The 6,300–6,400 A band always disappears, while that between 6,150 and 6,300 A persists. A rough indication of the amount of the two substances present is made by finding the dilution at which these bands disappear. The sulphæmoglobin is the more persistent, often being visible in a dilution of 1 in 30 to 1 in 50, while the band of methæmoglobin rarely persists above 1 in 10. Cyanosis appears to be rare after the administration of uleron, although Schubert (1937) has recorded cases with DB 87 (*cf.* p. 368); it is not uncommon with 2(*p*-aminobenzene-sulphonamido) pyridine.

Although Frost (1937) reported a fatal case of a child who developed sulphæmoglobinæmia and died of respiratory failure, cyanosis can usually be ignored provided it is not associated with severe anæmia, marked dyspnœa or cardiac failure.

Wendel (1937) recommends the use of methylene blue in cases of methæmoglobinæmia, a single injection of 1 mgm. per kilogram of body weight producing a very great reduction in cyanosis. In severe cases of cyanosis resort must be had to blood transfusion : oxygen is of little value.

References

ARCHER, H. E., and DISCOMBE, G., 1937, Sulphæmoglobinæmia, its cause and prevention, with special reference to treatment with sulphanilamide. *Lancet*, *ii.*, 432.

CHESLEY, L. C., 1938, Cyanosis without sulf- or methemoglobinemia in patients receiving sulfanilamide treatment. *J. clin. Invest.*, XVII., 445.

COKKINIS, A. J., 1937, Treatment of gonorrhœa with oral sulphanilamide : technique, toxic effects and early results in 250 cases. *Brit. med. J.*, *ii.*, 905.

Colebrook, L., and Kenny, M., 1936, Treatment of human puerperal infections, and of experimental infections in mice, with prontosil. *Lancet*, *i.*, 1279.

Colebrook, L., and Purdie, A. W., 1937, Treatment of 106 cases of puerperal fever by sulphanilamide (streptocide). *Ibid.*, *ii.*, 1237 and 1291.

Discombe, G., 1937, Sulphæmoglobinæmia following sulphanilamide treatment. *Ibid.*, *i.*, 626.

Frost, L. D. B., 1937, Sulphæmoglobinæmia following antistreptococcal chemotherapy. *Ibid.*, *i.*, 510.

Gley, P., 1937, Action sur le sang des dérivés de l'aniline (methémoglobinèmie et sulfhémoglobinèmie). *Bull. Acad. Méd., Paris*, CXVIII., 377.

Hewer, C. L., 1938, Sulfanilamide and pentothal. *Lancet*, *i.*, 1084.

Marshall, E. K., Jr., and Walzl, E. M., 1937, On cyanosis from sulfanilamide. *Bull. Johns Hopk. Hosp.*, LXI., 140.

Mull, J. W., and Smith, J. T., 1938, Effect of sulfanilamide on the oxygen capacity of the blood. *J. Amer. med. Ass.*, CX., 439.

Ottenberg, R., and Fox, C. L., Jr., 1938, Explanation of the cyanosis of sulphanilamide therapy. *Proc. Soc. exp. Biol., N.Y.*, XXXVIII., 479.

Paton, J. P. J., and Eaton, J. C., 1937, Sulphæmoglobinæmia and methæmoglobinæmia following administration of *p*-aminobenzenesulphonamide. *Lancet*, *i.*, 1159.

Peters, B. A., and Havard, R. V., 1937, Chemotherapy of streptococcal infections with *p*-benzylaminobenzenesulphonamide. *Ibid.*, *i.*, 1273.

Schubert, M., 1937, Zur Chemotherapie der Gonorrhöe mit Uliron (DB 90) und Diseptal B (DB 87). *Dermat. Wschr.*, CV., 1549.

Wendel, W. B., 1937, Use of methylene blue in methæmoglobinæmia from sulfanilamide poisoning. *J. Amer. med. Ass.*, CIX., 1216.

OTHER TOXIC SEQUELÆ

A small number of cases of what appears to be acute hepatitis have been described. Alstead (1937) reported one such case associated with jaundice, methæmoglobinæmia and a neutrophil leucocytosis, while Saphirstein's case (1938) exhibited a maculopapular rash, toxic hepatitis and bile in the urine. Some evidence has been obtained that liver function is depressed by therapeutic doses of sulphanilamide. Stümpke (1938) recorded enteritis following uleron administration. Dozzi (1938) has described transient nodal rhythm.

A case of nephritis attributed to the action of rubiazol has been reported by Hanns and Manganey (1937), while urinary irritation has also been recorded as a result of the administration of sulphanilamide. Jaubert and Motz (1938) allege that following the use of sulphanilamide in gonorrhœa there occurs a decrease in the number of spermatozoa produced and an increase in abnormal forms. Investigations in adult rabbits by Palazzoli *et al.* (1938) have failed to show that sulphanilamide has any effect on the fecundity of males or on the morphology of the spermatozoa. Levaditi and Vaisman (1938) obtained similar results in mice. If, however, young growing mice were repeatedly given sulphanilamide or uleron their growth was arrested, the amount of seminal fluid was decreased and the number of spermatozoa reduced. The effect is almost certainly non-specific.

References

Alstead, G., 1937, Toxiske Virkninger af aminobenzolsulfonamid (prontosil, streptamid, sulphanilamid). *Ugeskr. Laeg.*, **XCIX.**, 1347.

Dozzi, D. L., 1938, Transient nodal rhythm following use of sulfanilamide. *Amer. J. med. Sc.*, **CXCIX.**, 771.

Hanns, A., and Manganey, L., 1937, Néphrite par rubiazol. *Strasbourg-méd.*, **XCVII.**, 148.

Jaubert, A., and Motz, C. (1938). Action inhibitrice de certains dérivés organiques du soufre sur la spermatogenèse de l'homme. *Bull. Soc. franç. Urol.*, **LIV.**, 60.

Levaditi, C., and Vaisman, A., 1938, Dérivés benzéniques sulfamidés et spermatogenèse. Etude expérimentale. *C. R. Soc. Biol., Paris*, **CXXVIII.**, 352.

Palazzoli, M., Nitti, F., Bovet, D., and Levinson, M., 1938, Recherches expérimentales concernant l'action du *p*-aminophénylsulfamide sur la spermatogenèse du lapin. *Ibid.*, **CXXVIII.**, 261.

Saphirstein, H., 1938, Hepatitis and toxic erythema with desquamation due to sulfanilamide. *Urol. and Cut. Rev.*, **LXII.**, 101.

Stümpke, G., 1938, Nochmals zur Ulironfrage. *Dtsch. med. Wschr.*, **LXIV.**, 785.

THE PREVENTION OF TOXIC REACTIONS

As the cause of many of the reactions following the use of sulphonamide drugs is as yet little understood it is impossible to prescribe specific measures for their avoidance. Severe anæmia and leucopenia, marked renal impairment and obstructive jaundice, however, should be taken as contra-indications to the administration of drugs of the sulphonamide group. All foods containing sulphur should be avoided, in addition to drugs such as saline purges and iron sulphate. Putrefaction, and hence the development of sulphæmoglobinæmia, may be prevented, or at any rate delayed, by the use of a low residue diet of adequate caloric value, by cleansing the colon with enemata before treatment if saline purges have previously been given, and by administering regularly large doses of liquid paraffin. Spectroscopic examination of the blood, total red cell and total and differential leucocyte counts should be carried out at frequent intervals. Patients should also avoid exposure to natural sunlight or to ultra-violet light during treatment, and, above all, the drugs should not be given for more than fourteen, and preferably for not more than ten, consecutive days. It is important to remember that by increasing the fluid intake excretion of the drugs in the urine may be considerably increased with rapid disappearance of toxic symptoms. Addition of vitamin B_1 to the diet may possibly prevent the development of peripheral neuritis.

MODE OF ACTION

Before discussing the various theories which have been put forward to explain the mode of action of sulphonamide and other related compounds on bacterial infections, there are certain questions which require brief mention.

Correlation between Chemical Structure and Therapeutic Action ***in vivo.*** So far as sulphonamide compounds are concerned it appears to be essential for therapeutic efficiency that the —NH group in the benzene ring should occupy the *para*-position in relation to the —SO_2NH_2 group. While substitution in the amino group by acyl substituents leads to decrease in activity, substitution of other groups leads to either no change or slightly decreased activity. Substitution in the sulphonamide group on the other hand does not necessarily lead to decreased activity, disulphanilamide, for instance, being quite as active as, if not more so than, sulphanilamide in experimental streptococcal infections.

In the case of sulphone compounds, substitution in either amino group of acyl substituents produces reduced toxicity and, for streptococcal infections, little decrease in therapeutic activity.

Variation in Susceptibility of Different Bacteria. Experimental and clinical observations have shown that a number of different species of bacteria are susceptible to the action of sulphonamide compounds. Certain of these bacterial species show wide variations in general characteristics, yet organisms more closely allied, such as *Streptococcus viridans* and *Str. hæmolyticus*, show much diversity in their susceptibility to sulphonamide compounds. A similar difference is, however, shown in the reactions of these two organisms to other antiseptics, *Str. viridans* being as a rule the more resistant. Even among hæmolytic streptococci those belonging to Group A are probably more susceptible than those of other groups. A similar variation in the susceptibility of strains is noticeable in the case of the gonococcus. In testing the action of drugs on bacteria, hæmolytic streptococci of Group A may be taken as test organisms, since every compound of the sulphonamide series so far found to be active in any bacterial infection is active on these organisms: several strains of Group A hæmolytic streptococci were found by Buttle, Gray and Stephenson (1936) to be equally susceptible to sulphanilamide. The fact that a compound is active against streptococci is, however, no proof that it possesses any activity against pneumococci or even meningococci.

The Active Component of the Azo Dyes. In view of the fact, originally demonstrated by Domagk (1935), that despite its activity *in vivo* prontosil has only a very slight action on streptococci *in vitro*, it is now generally agreed that the active component of the azo-dyes must be split off in the tissues. When it had been demonstrated by Kellner (1936) and Fuller (1937) that *p*-aminobenzenesulphonamide is liberated in the tissues from prontosil and possesses considerable activity both *in vivo* and *in vitro* against streptococci, it was generally accepted, both in England and America, that *p*-aminobenzenesulphonamide is the bactericidal substance active in the body. On the Continent, however, the view is still held that prontosil *per se* may have some activity in the tissues or that it may be converted into some substance other than *p*-aminobenzenesulphonamide. Levaditi (1937) has postulated such an X substance.

The arguments adduced by Domagk (1936, 1937[1, 2]) and Hoerlein (1937) may be briefly summarised as follows:—

(i.) Since up to 95 per cent. of sulphanilamide given by mouth is excreted, either as such or in the relatively inactive acetylated form, large quantities cannot be retained in the body to form chemical combinations.

(ii.) Prontosil soluble, as shown by Rosenthal *et al.* (1937), is very rapidly excreted, yet injections of prontosil soluble given to mice infected with streptococci at twenty-four-hour intervals, treatment beginning one hour after infection, prevent fatal infections. In the treated mice peritoneal smears are almost normal, while smears from controls are full of cocci and phagocytic cells. If the activity of prontosil soluble depended on the sulphanilamide derived from it, in view of the rapidity of excretion such therapeutic activity is remarkable.

(iii.) Weight for weight prontosil is almost as effective *in vivo* as sulphanilamide.

(iv.) Of two azo-dyes, one is more active than the other, but both yield the same amount of sulphanilamide (Levaditi and Vaisman, 1937 [2]).

(v.) A number of other compounds, including the sulphones, are now known to be active against bacterial infections *in vivo.*

This last argument carries little weight, since no one believes that *p*-aminobenzenesulphonamide is the one and only compound formed in the tissues which is endowed with bactericidal activity.

In addition, up to the present all efforts to demonstrate the formation of an X substance either *in vivo* or *in vitro* have failed. Mayer (1937), however, has suggested that *para*-hydroxylaminophenylsulphonamide, which is therapeutically active, may represent such a substance. This compound is formed by the reduction of *p*-nitrobenzenesulphonamide. Since, however, *p*-aminobenzenesulphonamide is formed from prontosil, not only *in vivo* but also *in vitro*, by reduction with magnesium powder (Colebrook, Buttle and O'Meara, 1936), and from prontosil soluble by reduction with cysteine hydrochloride (Bliss and Long, 1937 [1]), it seems reasonable to regard it as the chief therapeutically active substance obtainable from prontosil until such time as positive evidence is brought forward that some other substance of even greater therapeutic activity is produced from prontosil.

Up to the present attention has been concentrated especially on the way in which sulphanilamide overcomes bacterial infections in the body, and as a result a number of theories have been put forward. These theories, which may be divided into two main divisions dependent on whether the main action of the drug is regarded as being directed against the bacteria or the cell defences of the body, are classified as follows :—

(I.) Action on bacteria.

- (*a*) bacteriostatic and bactericidal ;
- (*b*) interference with capsule formation ;

  - (*c*) neutralisation of toxins or inhibition of toxin formation.
- (II.) Action on phagocytic cells—
  - (*a*) mobilisation of macrophages ;
  - (*b*) increased phagocytosis.

Various combinations of these theories have also been suggested, as, for instance, that interference with capsule formation increases the possibilities of phagocytosis.

Bacteriostatic and Bactericidal Experiments. Incidental reference has already been made to a number of observations on the bacteriostatic or bactericidal action of sulphonamide drugs. The first actual observations on the action of sulphanilamide *in vitro* were those of Colebrook, Buttle and O'Meara (1936), who showed that in ordinary culture media a 1 in 10,000 concentration of sulphanilamide could delay the growth of 30 to 50 streptococci for from two to five days. In human blood a concentration of no more than 1 in 18,000 sulphanilamide, less, that is to say, than that attainable in the blood, could inhibit the growth of some hundreds or thousands of streptococci per c.c. This action was most marked in blood, more especially in human and monkey blood, and to a less extent in mouse or rabbit blood. It was also noticeable in serum and to a less extent in broth. These conditions explain why certain observers such as Bürgers (1937) and Domagk (1937[1, 2]) have failed to detect any definite bactericidal action *in vitro*, while others such as Finklestone-Sayliss, Paine and Patrick (1937) and Nitti, Bovet and Depierre (1937) have obtained positive results. In this connection, Finklestone-Sayliss, Paine and Patrick (1937) made the interesting observation that the bacteriostatic action of sulphanilamide on hæmolytic streptococci is preceded by a phase of growth stimulation. The growth stimulation is more conspicuous in young cultures than in cultures that have passed through the logarithmic phase of growth. Sulphanilamide is more soluble in the fatty envelope of hæmolytic streptococci than in aqueous solution, and a possible explanation of the bacteriostatic action is that, as age increases, the concentration of sulphanilamide in the fat of each individual organism increases.

Colebrook and Kenny (1936) found that, despite absence on the part of prontosil of bacteriostatic power *in vitro*, the serum of patients treated with this dye developed bactericidal properties, while Colebrook, Buttle and O'Meara (1936) showed that sulphanilamide conferred bactericidal properties on the blood *in vivo*. Hoare (1938) found that the blood may become bactericidal within one hour of administration of sulphanilamide or after a delay of a day or two, the difference being attributable to variation in the rate of absorption. The bactericidal action of the blood was not due to what has been termed "the Tillett" (1937) "phenomenon," that is to say, to a bactericidal power exerted on streptococci by the serum of persons suffering from streptococcal infections, but to some agent in the serum. Substitution experiments, in which leucocytes from healthy persons were used in place of the patient's own, showed that no change in the leucocytes of treated patients was in any way responsible. Although sulphanilamide was bactericidal *in vitro* in serum alone, the optimum effect was obtained with a concentration of 1 in 8,000, whereas in blood the optimum was 1 in 16,000, with lesser effects in each case up to one-eighth of these concentrations. Both Bliss and Long (1937[2]) and Hoare (1938) noted a close correlation between the bactericidal action of sulphanilamide on strains of hæmolytic streptococci *in vitro* and the reaction of these strains to sulphanilamide treatment *in vivo*. Felke (1937) has reported a similar correlation among strains of gonococci. These observations strongly support the view that *in vivo* sulphanilamide acts directly on hæmolytic streptococci, either killing them outright or at least preventing their growth; as Garrod (1938) points out, the limited numbers on which the drug acts *in vitro* correspond to those found in the blood-stream and elsewhere in widespread infection. Other observers, however, have suggested that *in vivo* bacteriostatic action is ancillary to the operation of the cellular defence mechanism of the body (Gay and Clark, 1937; McKinney and Mellon, 1937; Long and Bliss, 1937[1,2]; Bliss and Long, 1937[3]; and Osgood and Brownlee, 1938[1,2]). Levaditi and Vaisman (1938[3]) have attempted to show that in the body the bacteriostatic action of the sulphonamide drugs is negligible, whatever their *in vitro* action. They suggest an analogy with acriflavine, which *in vitro* inactivates streptococci in a dilution of 1 : 1,280,000, but injected intra-peritoneally delays the death of mice for only a very short period.

In an effort to determine whether any particular enzymes are attacked by the sulphonamide group of drugs, Mellon and Bambas (1937) have examined the dehydrogenase system and have found that, unlike sodium glycocholate and optochin, sulphanilamide does not interfere with these enzymes, at any rate in the case of pneumococcus. While prontosil itself had no action on the metabolism of hæmolytic streptococci, *Bact. coli*, Friedlander's bacillus or the gonococcus, sulphanilamide was found by Barron and Jacobs (1937) to have a slightly inhibitory effect on the oxidation of glucose by hæmolytic streptococci and on the oxidation of glucose and lactose by Friedlander's bacillus.

Gley and Girard (1937) believe that exposure of streptococci to prontosil or sulphanilamide *in vitro* decreases their virulence. This finding awaits confirmation.

Inhibition of Capsule Formation. Another view, originally put forward by Levaditi and Vaisman (1935[1]), is that the sulphonamide group of drugs interferes with capsule formation in the streptococci and pneumococci (Levaditi, Vaisman and Krassnoff, 1938[1]). Levaditi (1938[1, 2]) has failed to convince himself that sulphanilamide has a bacteriostatic action on streptococci, but believes rather that the failure to produce capsules, combined with inhibition of toxin formation, leads to greater phagocytosis. In the case of the pneumococcus, Levaditi, Vaisman and Krassnoff (1938[2]) have demonstrated that pneumococci deprived of their capsules are readily phagocytosed in the peritoneal cavity of the mouse. Immune serum and the sulphonamide group of drugs, it is claimed, " decapsulise " the pneumococci and thus render them easily phagocytosed.

In this connection it is interesting to note that when pneumococci are acted on by 2(*p*-aminobenzenesulphonamido) pyridine their capsules swell up and disappear (Whitby, 1938). Similar observations were made by Telling and Oliver (1938) in the treatment of a patient with a massive pneumonia due to pneumococcus Type III. After the patient had received 3 gm. of 2(*p*-aminobenzenesulphonamido) pyridine the pneumococci obtained were scanty, pleomorphic and non-capsulated : primary culture yielded a pure atypical growth not agglutinated by Type III or any other serum, and mouse inoculation produced an exudate showing no agglutination and no capsular swelling or staining. After two passages in mice this strain of the pneumococcus became typical of Type III again, but another strain isolated from the patient required further passage before it returned to type. Similarly, a Type XI pneumococcus isolated from a patient with broncho-pneumonia also lost its capsule and its specific agglutinability, while in a patient with a Type I pneumonia the organisms rapidly disappeared from the sputum.

The fact that immune serum apparently acts in the same way as sulphonamide drugs is of interest, since Meyer (1938) has found that certain patients who have failed to respond to sulphanilamide in a satisfactory manner are cured by additional treatment with anti-toxic streptococcus serum. Similarly, in the treatment of gonorrhœa, uleron appears to act better on cases which have acquired some degree of immunity to the infection.

Neutralisation of Toxins. Reference has already been made to the view put forward by Levaditi and Vaisman (1935[2]) to the effect that sulphanilamide neutralises the hæmolysins and leucocidins of streptococci. Meyer (1937) has confirmed these results, while Osgood and Brownlee (1938[1]) believed in the absence of hæmolysins in streptococcus cultures containing sulphanilamide, since bone marrow cells exposed to the streptococci retained their morphology. The view that sulphonamide compounds neutralise or inhibit the formation of endotoxins in other organisms has been developed by Levaditi and his colleagues in relation to the endotoxins of the gonococcus and the action of 4-nitro-4′-amino-diphenylsulphoxide (Levaditi and Vaisman, 1937[2]; Levaditi, Vaisman and Reinié, 1937), and the endotoxins of the meningococcus, *Bact. aertrycke* and *Pasteurella avicida*, with the same compounds (Levaditi and Vaisman, 1938[1]). These compounds do not neutralise the exotoxins of the staphylococcus.

Sulphanilamide and 4-nitro-4′-aminodiphenylsulphoxide were also thought to neutralise the endotoxins of *Bact. dysenteriæ* Shiga and Flexner *in vivo* but not *in vitro* (Levaditi and Vaisman, 1938 [2]). Osgood and Brownlee (1938[1]) believed that *in vitro* sulphanilamide inactivated a certain fraction of freshly prepared *Bacillus perfringens* hæmotoxin, but did not neutralise diphtheria toxin or tetanus neuro-toxin.

These views of the action of sulphanilamide and related compounds have been subjected to considerable criticism. Thus Gross, Cooper and Lewis (1938) found that prontosil itself had no inhibitory effect on streptococcal hæmolysins; sulphanilamide caused a very slight, almost negligible, inhibition of the action of streptococcal and staphylococcal hæmotoxins, an inhibition more in evidence with prontosil soluble and a variety of other substances such as sodium carbonate, phenol, mercuric chloride, normal rabbit serum or rabbit serum heated to 61° C. The administration of sulphanilamide to a rabbit or its addition to rabbit serum *in vitro* did not enhance the inhibitory effect of rabbit serum on streptococcal hæmolysins. Huntington (1938) and Kemp (1938) similarly found that *in vitro*, in concentrations equal to or greater than those therapeutically induced in the body fluids, sulphanilamide was without effect upon the hæmolysin, fibrinolysin or erythrogenic toxin formation of streptococci. Garrod (1938) also observed no neutralisation of Dick toxin *in vitro*. In other words, it seems that the inhibitory action of sulphanilamide on toxin formation is due to the inhibitory effect on the growth of the micro-organisms and is, in fact, an effect given by many disinfectant substances when present in concentrations insufficient to prevent growth.

The Action of Sulphonamide Compounds on Phagocytic Cells. While there is now evidence in favour of the view that, at any rate in the case of the pneumococcus, certain sulphonamide compounds act as decapsulating agents, and also evidence in favour of a direct bactericidal or at least basteriostatic action, independent of the leucocytes, the evidence of any direct stimulatory action on the phagocytic cells of the body is somewhat meagre. Levaditi and Vaisman (1935[3]) noted a macrophage increase in mice treated with prontosil, but blockade with colloidal copper did not decrease the chemotherapeutic action of the drug. Gay and Clark (1937), in studying the effects of sulphanilamide in experimental streptococcal empyema, believed that co-operation on the part of locally derived clasmatocytes was required for complete sterilisation. Long and Bliss (1937[2]), who examined smears from the peritoneum of mice infected with streptococci and then given sulphanilamide, found that for from six to fifteen hours the organisms multiplied, retaining their capsules, then suddenly a wave of phagocytes appeared and the organisms were ingested. Finklestone-Sayliss, Paine and Patrick (1937) believed that in the rabbit the activity of the reticulo-endothelial cells is stimulated by sulphanilamide, while Levaditi, Bequignon and Reinié (1938) showed that when given by mouth the coloured sulphonamide drugs localised in tissue granulomata produced by a variety of agents. On the other hand, Domagk (1937[1]) found that the peritoneal cavity in infected mice treated with prontosil appeared almost normal in a few hours, while Mellon, Gross and Cooper (1937) noted little evidence of increased phagocytosis either in smears from the peritoneal exudate or in sections of liver and spleen from mice infected with streptococci and treated with sulphanilamide. On the other hand, Finkelstein and Birkeland (1938) believe that *in vitro* it is possible to demonstrate that sulphanilamide enhances the phagocytic power of the leucocytes against hæmolytic streptococci, provided serum or plasma are present, the effect varying with the concentration of the drug :—

THE EFFECT OF VARYING DILUTIONS OF SULPHANILAMIDE ON PHAGOCYTOSIS *IN VITRO*

Dilution of drug.	No. of cocci phagocytosed.	Percentage of leucocytes acting as phagocytes.
in 1,000	192	20 per cent.
,, 10,000	246	32 ,, ,,
,, 50,000	514	60 ,, ,,
100,000	296	48 ,, ,,
250,000	82	12 ,, ,,
Control without drug	96	16 ,, ,,

A serum-sulphanilamide complex thus acts, it is suggested, as an opsonin, rendering the bacteria more readily phagocytable. This view is somewhat akin to that of Welch, Wentworth and Mickle (1938), who find that leucocytes obtained from human beings infected with *Brucella abortus* and treated with sulphanilamide acquire an enhanced power to phagocytose *Br. abortus*. The blood of normal human beings and guinea-pigs, when treated *in vitro* with sulphanilamide, shows no change in opsonic power either against normal *Br. abortus* or against the same organisms mixed with sulphanilamide. If, however, normal leucocytes are added to the serum of a guinea-pig, which after infection with *Br. abortus* has received sulphanilamide, then the increased phagocytic activity of the leucocytes becomes apparent. It is therefore suggested that sulphanilamide acts by neutralising the endotoxins or aggressins formed by *Br. abortus*, thereby increasing the opsonic power of the leucocytes. This indirect action is, however, brought into play only when an infective or, more correctly, an immunising process is already taking place in the tissues. *In vitro* Coman (1938) has failed to show that prontosil and allied compounds have any action in enhancing the chemotropism of leucocytes, nor do they influence the temperature or leucocyte response which follow injection of turpentine (Unshelm and Hofmann, 1937). The effect of prontosil and prontosil soluble on the isoelectric point of egg albumen has been studied by Soehring (1937), but little light is thrown on the mode of action of the drugs.

References

Barron, E. S. G., and Jacobs, H. H., 1937, Effect of prontosil and prontylin on metabolism of bacteria. *Proc. Soc. exp. Biol., N.Y.*, XXXVII., 10.

Bliss, E. A., and Long, P. H., 1937[1], Activation of " prontosil solution " *in vitro* by reduction with cysteine hydrochloride. *Johns Hopk. Hosp. Bull.*, LX., 149.

Bliss, E. A., and Long, P. H., 1937[2], Failure of para-aminobenzenesulfonamide therapy in urinary tract infections due to group D (Lancefield) beta-hemolytic streptococci. *New Engl. J. Med.*, CCXVII., 18.

Bliss, E. A., and Long, P. H., 1937[3], Observations on the mode of action of sulfanilamide. *J. Amer. med. Ass.*, CIX., 1524.

Bürgers, 1937, Chemotherapie der Streptokokkenerkrankungen durch Prontosil. *Dtsch. med. Wschr.*, LXIII., 672.

BUTTLE, G. A. H., GRAY, W. H., and STEPHENSON, D., 1936, Protection of mice against streptococcal and other infections by *p*-aminobenzenesulphonamide and related substances. *Lancet*, *i.*, 1286.

COLEBROOK, L., BUTTLE, G. A. H., and O'MEARA, R. A. Q., 1936, The mode of action of *p*-aminobenzenesulphonamide and prontosil in hæmolytic streptococcal infections. *Ibid.*, *ii.*, 1323.

COLEBROOK, L., and KENNY, M., 1936, Treatment of human puerperal infections and of experimental infections in mice with prontosil. *Ibid.*, *i.*, 1279.

COMAN, D. R., 1938, The effect of prontosil and related compounds upon the chemotropism of leukocytes. *Amer. J. med. Sci.*, CXCVI., 273.

DOMAGK, G., 1935, Ein Beitrag zur Chemotherapie der bakteriellen Infektionen. *Dtsch. med. Wschr.*, LXI., 250.

DOMAGK, G., 1936, Chemotherapie der Streptokokkeninfektionen. *Klin. Wschr.*, XV., 1585.

DOMAGK, G., 1937 [1], Weitere Untersuchungen über die chemotherapeutische Wirkung sulfonamidhaltiger Verbindungen bei bakteriellen Infektionen. *Ibid.*, XVI., 1412.

DOMAGK, G., 1937 [2], Untersuchungen über den Wirkungsmechanismus des Prontosil bei der experimentellen Streptokokkeninfektion. *Z. klin. Med.*, CXXXII., 775.

FELKE, H., 1937, Die Wirkung der Sulfonamidverbindungen auf die Erreger der Gonorrhöe. *Klin. Wschr.*, XVII., 13.

FINKELSTEIN, R., and BIRKELAND, J. M., 1938, The mode of action of sulfanilamide and prontosil. *Science*, LXXXVII., 441.

FINKLESTONE-SAYLISS, H., PAINE, C. G., and PATRICK, L. B., 1937, Bacteriostatic action of *p*-aminobenzenesulphonamide upon hæmolytic streptococci. *Lancet*, *ii.*, 792.

FULLER, A. T., 1937, Is *p*-aminobenzenesulfonamide the active agent in prontosil therapy ? *Ibid.*, *i.*, 194.

GARROD, L. P., 1938, The chemotherapy of bacterial infections. *Ibid.*, *i.*, 1125 and 1178.

GAY, F. P., and CLARK, A. R., 1937, On the mode of action of sulfanilamide in experimental streptococcus empyema. *J. exp. Med.*, LXVI., 535.

GLEY, P., and GIRARD, A., 1937, Sur le mécanisme de l'action anti-streptococcique de la sulfamidochrysoidine. *Pr. méd.*, XLV., 1291.

GROSS, P., COOPER, F. B., and LEWIS, M., 1938, Inhibition of streptococcal hemolysin by sulfonamide compounds. *Proc. Soc. exp. Biol., N.Y.*, XXXVIII., 275.

HOARE, E. D., 1938, Bactericidal changes induced in human blood and serum by sulphamido-chrysoidine and sulphanilamide. *Lancet*, *i.*, 655.

HOERLEIN, H., 1937, The development of chemotherapy for bacterial diseases. *Practitioner*, CXXXIX., 635.

HUNTINGTON, R. W., Jr., 1938, Failure of sulfanilamide to prevent hemolysis, fibrinolysis and production of erythrogenic toxin by hemolytic streptococci *in vitro*. *Proc. Soc. exp. Biol., N.Y.*, XXXVIII., 328.

KELLNER, K., 1936, Etude sur l'élimination de la sulfamido-chrysoïdine. Thèse, Paris.

KEMP, H. A., 1938, Action of sulfanilamide. Failure to demonstrate antihemolytic, antifibrinolytic and antitoxic effect of the drug. *Texas State J. Med.*, XXXIV., 208.

LEVADITI, C., 1937, " Le mode d'action des dérivés benzéniques sulfurés dans les infections microbiennes expérimentales." Monographs of the Institut Alfred Fournier, Paris, No. 5.

LEVADITI, C., 1938 [1], Mode d'action des médicaments sulfurés dans les infections provoquées par le streptocoque, le pneumocoque, le meningocoque et le gonocoque. *Paris méd.*, **XXVII.**, 189.

LEVADITI, C., 1938 [2], Réflexions sur le passé, le présent et l'avenir de la chimiothérapie des maladies microbiennes. *Pr. méd.*, **XLVI.**, 572.

LEVADITI, C., BEQUIGNON, R., and REINIÉ, L., 1938, Affinités tissulaires des azoïques sulfamidés. *C. R. Soc. Biol., Paris*, **CXXVIII.**, 355.

LEVADITI, C., and VAISMAN, A., 1935 [1], action curative du chlorhydrate de 4^1-sulfamido-2 : 4-diaminoazobenzène et de quelques dérivés similaires, dans la streptococcie expérimentale. *Ibid.*, **CXIX.**, 946.

LEVADITI, C., and VAISMAN, A., 1935 [2], Mécanisme de l'action curative et préventive du chlorhydrate de 4^1-sulfamido-2 : 4-diaminoazobenzène et d'autres dérivés similaires dans l'infection streptococcique expérimentale. *Ibid.*, **CXX.**, 1077.

LEVADITI, C., and VAISMAN, A., 1935 [3], Action curative et préventive du chlorhydrate de 4^1-sulfamido-2, 4-diaminoazobenzène et de quelques dérivés similaires dans la streptococcie expérimentale. *Pr. méd.*, **XLIII.**, 2097.

LEVADITI, C., and VAISMAN, A., 1937 [1], Chimiothérapie de l'infection méningococcique expérimentale de la souris. *C. R. Soc. Biol., Paris*, **CXXV.**, 604.

LEVADITI, C., and VAISMAN, A., 1937 [2], Chimiothérapie anti-endotoxique. *Ibid.*, **CCV.**, 1108.

LEVADITI, C., and VAISMAN, A., 1938 [1], Chimiothérapie antiendotoxique. Endotoxine de la *Pasteurella avicida*. *Ibid.*, **CXXVIII.**, 283.

LEVADITI, C., and VAISMAN A., 1938 [2], La chimiothérapie antiendotoxique et son mécanisme. *Ibid.*, **CXXVIII.**, 463.

LEVADITI, C., and VAISMAN, A., 1938 [3], Au sujet du mécanisme de l'action thérapeutique des dérivés benzéniques sulfurés dans l'infection streptococcique expérimentale. *Ibid.*, **CXXVIII.**, 476.

LEVADITI, C., VAISMAN, A., and KRASSNOFF, D., 1938 [1], Mécanisme de la chimiothérapie antipneumococcique. *Ibid.*, **CXXVII.**, 22.

LEVADITI, C., VAISMAN, A., and KRASSNOFF, D., 1938 [2], Mécanisme de l'action chimiothérapique des dérivés à base de soufre dans les pneumococcies expérimentales. *Bull. Acad. Méd., Paris*, **CXIX.**, 553.

LEVADITI, C., VAISMAN, A., and REINIÉ, L., 1937, Chimiothérapie antiendotoxique. *C. R. Soc. Biol., Paris*, **CXXVI.**, 1093.

LONG, P. H., and BLISS, E. A., 1937 [1], Para-aminobenzenesulfonamide and its derivatives : experimental and clinical observations on their use in treatment of beta hemolytic streptoccic infection : preliminary report. *J. Amer. med. Ass.*, **CVIII.**, 32.

LONG, P. H., and BLISS, E. A., 1937 [2], Use of para-aminobenzenesulphonamide (sulfanilamide) or its derivatives in treatment of infections due to beta hemolytic streptococci, pneumococci and meningococci. *Sth. med. J.*, **XXX.**, 479.

MCKINNEY, R. A., and MELLON, R. R., 1937, Sulfanilamide and the macrophage response to hemolytic streptococcal peritonitis in mice. *Proc. Soc. exp. Biol., N.Y.*, **XXXVII.**, 333.

MAYER, R. L., 1937, Recherches sur le mécanisme de l'action anti-streptococcique de l'aminobenzènesulfamide et de ses dérivés. *Bull. Acad. Méd., Paris*, **CXVII.**, 727.

MELLON, R. R., and BAMBAS, L. L., 1937, Bacteriostatic effect of sulfanilamide in spinal fluid of convalescent cases of streptococcal meningitis. *Proc. Soc. exp. Biol., N.Y.*, **XXXVI.**, 682.

MELLON, R., GROSS, P., and COOPER, F., 1937, Experimental studies with sulfanilamide and with prontosil in hemolytic streptococcus infections. *J. Amer. med. Ass.*, **CVIII.**, 1858.

MEYER, F., 1937, Streptococcus infection and the mechanism of its healing by serum and chemotherapy. *Quart. Bull. Sea View Hosp.*, II., 148.

MEYER, F., 1938, The mechanism of the new chemotherapy. *Brit. med. J.*, *i.*, 810.

NITTI, F., BOVET, D., and DEPIERRE, F., 1937, Action des dérivés du *p*-amino-phénylsulfamide (1162F) sur les streptocoques hémolytiques *in vitro*. *C. R. Soc. Biol., Paris*, CXIX., 1277.

OSGOOD, E. E., and BROWNLEE, I. E., 1938 [1], Culture of human marrow. Studies on the mode of action of sulfanilamide. *J. Amer. med. Ass.*, CX., 349.

OSGOOD, E. E., and BROWNLEE, I. E., 1938 [2], Culture of human marrow: a comparative study of the effects of sulfanilamide and antipneumococcus serum on the course of pneumococcic infections. *Arch. int. Med.*, LXII., 181.

ROSENTHAL, S. M., BAUER, H., and BRANHAM, S. E., 1937, Studies in chemotherapy. Comparative studies of sulphonamide compounds in experimental pneumococcus, streptococcus and meningococcus infections. *Publ. Hlth. Rep., Wash.*, LII., 662.

SOEHRING, K., 1937, Die “Eieweisswirkung” der Prontosilverbindungen. *Biochem. Z.*, CCXCV., 265.

TELLING, M., and OLIVER, W. A., 1938, Case of massive pneumonia, Type III, with massive collapse, treated with 2(*p*-aminobenzenesulphonamido) pyridine. *Lancet*, *i.*, 1391.

TILLETT, W. S., 1937, Bactericidal action of human serum on hemolytic streptococci: observations made with serum from patients with acute infections and from normal individuals. *J. exp. Med.*, LXV., 147.

UNSHELM, E., and HOFMANN, W., 1937, Zur Frage der Wirkungsmöglichkeit von Prontosil. *Z. ges. exp. Med.*, CCII., 43.

WELCH, H., WENTWORTH, J. A., and MICKLE, F. L., 1938, The use of sulfanilamide in the diagnosis and treatment of brucellosis. *J. Amer. med. Ass.*, CXI., 226.

WHITBY, L. E. H., 1938, Chemotherapy of pneumococcal and other infections with 2(*p*-aminobenzenesulphonamide) pyridine. *Lancet*, *i.*, 1210.

OTHER COMPOUNDS IN THE CHEMOTHERAPY OF BACTERIAL INFECTION

The stimulus given to chemotherapeutic research by the discovery of the sulphonamide drugs has resulted both in a search for entirely new compounds endowed with bactericidal action in the tissues and in a revaluation of drugs which were already known to exert some action on bacterial infections, however slight.

Thus, acriflavine and its derivatives, optochin and allied compounds have been re-investigated, while a somewhat miscellaneous collection of compounds has also been studied for chemotherapeutic action.

AURO-DETOXIN

" Auro-detoxin " and " Auro-detoxin Type 70 " are some of a number of water-soluble products containing metals prepared by means of keratin. Auro-detoxin contains 12 per cent. of metallic gold, and has been studied by Collier and Verhoog (1937) and Vaisman (1937) for its action in bacterial infections. Of auro-detoxin Type 70 a mouse of 20 gm. body weight can be given 0·15 gm. subcutaneously, while a rabbit shows no symptoms after the intravenous injection of 0·5 gm. per kilogram of body weight given as a 10 per cent. solution in distilled water. No action was noted on hæmolytic staphylococci, Friedlander's bacillus or *Bact. paratyphosus*, B. Eighty-five out of ninety mice infected with streptococci were cured by subcutaneous injections of 0·15 gm. for a 20 gm. mouse. A therapeutic action was also shown in mice infected with pneumococci Types I and III. Syphilis in the rabbit and infections due to *Spirochæta duttoni* are also said to be rapidly cured.

References

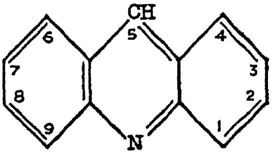
Collier, W. A., and Verhoog, M. J., 1937, Die Wirkung von Auro-Detoxin und Auro-Detoxin Type 70 auf verschiedene Infektionen. *Z. Immunforsch.*, XC., 174.

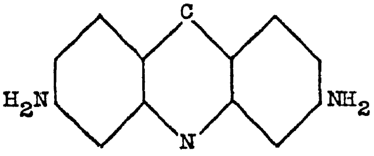
Vaisman, A., 1937, Action thérapeutique de l'auro-détoxine dans la streptococcie, la pneumococcie, la syphilis et la fièvre récurrente expérimentales. *C. R. Soc. Biol., Paris*, CXXIV., 1271.

ACRIDINE DERIVATIVES

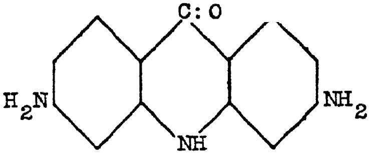
The earliest study of the relation between antiseptic action and chemical constitution among acridine derivatives was made by Browning *et al.* (1922), who showed that antiseptic action depended on an intact acridine nucleus, while the presence of an amino-substituent was necessary for the development of maximum effect. Of the many complicated substances derived from acridine, only rivanol (2 : 5-diamino-7-ethoxyacridine, *cf.* p. 85) has enjoyed a measure of success in chemotherapy, while the simple aminoacridines have been comparatively neglected. Of the twenty possible diaminoacridines only proflavine, Formula II, has been thoroughly investigated and has been employed not only as a local antiseptic, but also in the treatment of gonorrhœa and more recently meningococcal meningitis (Puig, 1937). Bogert *et al.* (1930), however, made some preliminary tests with 3 : 7-diamino-acridine and Morgenroth *et al.* (1921) carried out tests, restricted to streptococci, with the 2 : 5-isomeride.

Investigations were, therefore, made by Albert *et al.* (1938) to determine whether, with modification of the molecule of proflavine, Formula II, provided a single 2-amino group be retained, activity is susceptible to considerable change. With this end in view 2 : 8-diaminoacridone (Formula III), 2-amino-8-acetamidoacridine and 2-aminoacridine were prepared. In addition, Albert and Linnell (1936 and 1938) prepared the isomerides of proflavine to obtain information concerning the optimum position of the amino group or groups. As the two benzene rings in acridine are situated symmetrically with reference to the nitrogen atom in the central pyridine nucleus in Formula I, positions 2 and 8, 3 and 7, and so on, are chemically equivalent so that 2-aminoacridine is also 8-aminoacridine. Where two positions are substituted identity does not necessarily follow : thus the 2 : 8 positions are not identical as a pair with 3 : 7, but the two pairs 2 : 7- and 3 : 8- are identical, one component of each pair being in the same relative position to the central nitrogen atom. A point of some interest is that in 5-aminoacridine and 5-amino-2-chloroacridine no primary amino group appears to be present, and in the form of their salts, at least, they assume the tautomeric form illustrated by Formula IV, where 5-amino-2-chloroacridine is shown as the isomeric 2-chloroacridonimine.

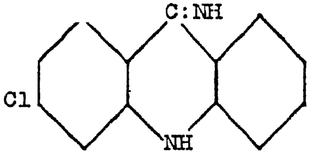




II



III



IV

The chief physical and biological properties of the acridine compounds examined for their bacteriostatic and bactericidal action on *Streptococcus pyogenes* are shown in the Table. The toxicities were obtained by Albert, Dyer and Linnell (1936).

The following conclusions may be drawn in regard to the use of these substances as chemotherapeutic remedies. 1-*Aminoacridines* are devoid of antiseptic activity even when a 2-amino group is also present. 2-*Aminoacridines* : The 2-amino substituent introduces marked antiseptic properties, which are further increased by the addition, in another ring, of a 5-, 6-, 7- or 8-amino group. This leads to increased toxicity only when the added substituent is *m*-amino group, giving proflavine. 2 : 8-Diaminoacridine : Acetylation of one amino group in the latter compound again leads to loss of activity. 3-*Aminoacridines* : 3 : 8-diaminoacridine is approximately equal to proflavine in bactericidal activity but less toxic. 4-*Aminoacridines* : 4 : 8-diaminoacridine

Physical and Biological Properties of Acridine Compounds (Albert *et al.*, 1938)

	Solubility in water at 15° C.	Solubility in physiological saline.	Lethal dose in mice per kilogm. of body weight	Maximum conc. allowing motility of leucocytes	Index of bacteriostatic capacity	Per cent. of *S. pyogenes* killed in 1 : 5,000 sol. in 2 hours
2-aminoacridine monohydrochloride	0·5%	rather less	0·35	1 in 8,400	36	30
2 : 5-diaminoacridine monohydrochloride	1·0%	0·4%	0·30	,, ,,	31	99·5–99·9
2 : 6-diaminoacridine hemisulphate	0·4%	slightly soluble	0·35	1 in 4,200	36	40
2 : 7-diaminoacridine monohydrochloride	1·0%	0·4%	0·50	1 in 2,100	34	92
2 : 8-diaminoacridine hemisulphate (proflavine)	0·5%	0·3%	0·20	,, ,,	40	93·7–97·3
2 : 9-diaminoacridine hemisulphate	1·0%	slightly soluble	0·75	,, ,,	4	not tested
2-amino-8-acetamidoacridine monohydrochloride	1·0%	slightly soluble	0·38	,, ,,	22	,, ,,
5-amino-2-chloroacridine monohydrochloride	over 2%	almost soluble	0·13	1 in 4,200	high	,, ,,
acriflavine . . .	very variable	0·2%	0·05	1 in 2,100	48	,, ,,

exhibited considerable activity. 5-*Aminoacridines* are highly bactericidal against streptococci, especially 5-amino-2-chloroacridine, but unfortunately its toxicity is increased.

The acridone nucleus does not share the antiseptic properties of the acridine nucleus. Unfortunately 2 : 6-diaminoacridine hemisulphate, 2 : 9-diaminoacridine hemisulphate, 2-amino-8-acetamidoacridine monohydrochloride and 5-amino-2-chloroacridine are almost insoluble, or only slightly soluble, in physiological saline, so that there is little possibility of their being used *in vivo*. 2 : 7-Diaminoacridine, however, is as innocuous to leucocytes as acriflavine and proflavine and possesses a bactericidal capacity approximately equal and a bacteriostatic capacity scarcely inferior to that of proflavine; it may possibly prove useful therapeutically. Its bacteriostatic action on *Staphylococcus aureus* and *Bacterium coli* was less than on *Streptococcus pyogenes*, while *Pseudomonas pyocyanea* was entirely insusceptible.

References

ALBERT, A., DYER, F. J., and LINNELL, W. H., 1936, Chemotherapeutic studies in the acridine series. Part IV. The relations between structure and toxicity. *Quart. J. Pharm.*, X., 649.

ALBERT, A., FRANCIS, A. E., GARROD, L. P., and LINNELL, W. H., 1938, Chemotherapeutic studies in the acridine series. The relation between chemical constitution and biological action in simple aminoacridines. *Brit. J. exp. Path.*, XIX., 41.

ALBERT, A., and LINNELL, W. H., 1936, Chemotherapeutic studies in the acridine series. Part I. 2 : 6- and 2 : 8-Diaminoacridines. Part II. 2-Amino, 2 : 5-, 2 : 7-, and 2 : 9-diamino-acridines. *J. chem. Soc.*, 88 and 1614.

ALBERT, A., and LINNELL, W. H., 1938, Chemotherapeutic studies in the acridine series. Part III. 4-Amino-, 1 : 3-, 1 : 7-, and 3 : 6-diamino-acridines. *Ibid.*, 22.

BOGERT, M. T., HIRSHFELDER, A. D., and LAUFFER, P. G. I., 1930, Researches in the acridine series. The synthesis of isomers of proflavine and neutral acriflavine. *Coll. Czech. Chem. Commun.*, II., 383.

BROWNING, C. H., COHEN, J. B., GAUNT, R., and GULBRANSEN, R., 1922, Relationships between antiseptic action and chemical constitution with special reference to compounds of the pyridine, quinoline, acridine and phenazine series. *Proc. roy. Soc.*, B, XCIII., 329.

MORGENROTH, J., SCHNITZER, R., and ROSENBERG, E., 1921, Ueber chemotherapeutische Antisepsis. III. Mitteilung. Ueber ein neues Antiseptikum (2-Aethoxy-6, 9-diaminoakridinchlorhydrat. *Dtsch. med. Wschr.*, XLVII., 1317.

PUIG, R., 1937, Le jaune d'acridine dans le traitement de la méningite cérébro-spinale. *Bull. Mém. Soc. méd. Hôp., Paris*, LIII., 1429.

PYRIDINE DERIVATIVES

Considerable interest has arisen in regard to the therapeutic activities of pyridine derivatives. Thus Bremer and Lippelt (1937) studied the bactericidal action of the following compounds : 3-nitro-4-butoxypyridine, 3-amino-4-butoxypyridine, 4-methoxy-pyridyl-3 : 5′-azo-2′ : 6′-diaminopyridine and 6-methoxy-quinolyl-3 : 5′-azo-2′ : 6′-diaminopyridine. Of these the most active was 4-methoxypyridyl-3 : 5′-azo-2′ : 6′-diaminopyridine, which killed *Bacterium coli* in a dilution of 1 in 100,000 after an exposure of three hours at 37° C. Reference (p. 369) has already been made to the sodium salt of *p*-sulphamidophenylpyridine-β-carboxyamido-α-carbonic acid which Hykes, Hykes and Rerabek (1937) have found to be active in streptococcal infections of mice, while 2(*p*-aminobenzenesulphonamido) pyridine, which is active also in experimental streptococcal infections of mice (*cf.* Whitby, 1938) is discussed on p. 386 in relation to the chemotherapy of pneumococcal infections.

Kolmer *et al.* (1937) prepared a number of pyridine compounds containing an amino group or amino and iodo groups, somewhat similar to the iodopyridine derivatives prepared by Binz and Maier-Bode (1933). The most promising compounds in the treatment of streptococcal infections of the rabbit were the thiocyanate of 2-aminopyridine and 2-amino-3-iodo-pyridine. 2-2′-Pyridyl sulphide dihydrobromide was also found to have an action very similar to that of sulphanilamide. The possibility of replacing the benzene nucleus by a pyridine nucleus, particularly with divalent sulphur in the molecule, thus appears worthy of further investigation.

As a urinary antiseptic, phenylazo-α : α-diaminopyridine hydrochloride (*Pyridium*) has been recommended (Neuburger, 1930). It may produce nausea, abdominal pains, headache and giddiness and should not be given in cases of nephritis, severe hepatitis or to patients with chronic gastro-intestinal derangement.

It appears to have no advantages over either mandelic acid or sulphanilamide in the treatment of either cystitis due to *Bact. coli* or of gonorrhœal infections (*cf.* Tatum, 1932).

References

Binz, A., and Maier-Bode, H., 1933, Die Wirkung von Iodpyridonderivaten auf Streptococcus. *Biochem. Z.*, **CCLVII.**, 351.

Bremer, O., and Lippelt, H., 1937, Sur Chemie und Bakteriologie neuer Pyridin-azo-Verbindungen. *Arch. Schiffs- u. Tropenhyg.*, **XLI.**, 737.

Hykes, O. V., Hykes, D. E., and Rerabek, J., 1937, Action spécifique d'un nouveau dérivé de la *p*-aminophénylsulfamide sur la septicémie streptococcique expérimentale. *C. R. Soc. Biol., Paris*, **CXXVI.**, 635.

Kolmer, J. A., Brown, H., Raiziss, G. W., Rule, A. M., and Clemence, L. W., 1937, Chemotherapy of experimental streptococcic infections of rabbits with especial reference to pyridine compounds and prontosil soluble. *J. Pharmacol.*, **LXI.**, 253.

Neuburger, J., 1930, Pyridium, ein neues Harndesinfiziens. *Münch. med. Wschr.*, **LXXVII.**, 1016.

Tatum, W. B., 1932, The use of pyridium in the treatment of genito-urinary infections in women and children. *Med. J. Rec.*, **CXXXVI.**, 207.

Whitby, L. E. H., 1938, Chemotherapy of pneumococcic and other infections with 2-(*p*-Aminobenzenesulphonamido)-pyridine. *Lancet*, *i.*, 1210.

QUININE DERIVATIVES AND PNEUMOCOCCUS

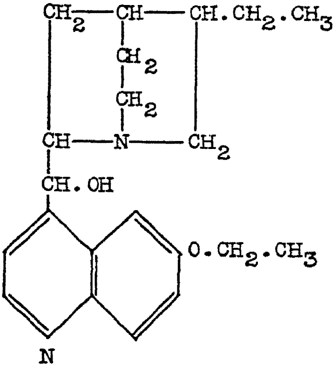
It has been long and generally believed that quinine possesses certain curative properties in pneumococcal infections and more especially in pneumonia. Kemkes (1937), for instance, claims that quinine glutamate and quinine lactate are pneumococcicidal in mice. In order to study this question, Morgenroth and Levy (1911) and Morgenroth and Bieling (1917) prepared, among other compounds, a series of derivatives of hydrocupreine or, more correctly, dihydrocupreine. The most interesting of these derivatives was ethyldihydrocupreine, which, under the name of optochin (optoquin), has been employed in the treatment of experimental pneumococcal infections in mice. The difference between the minimum lethal dose and the minimum curative dose is, however, too small to establish a definite therapeutic effect once the pneumococcal infection has become systemic. The use of optochin in pneumococcal infections in man has had extensive trials, but the results in lobar pneumonia have not been very striking (*cf.* Moore and Chesney, 1918, and Cohen, 1919).

In the treatment of eye diseases such as pneumococcal conjunctivitis, keratitis and dacrocystitis, optochin has been used with apparent success, being applied directly to the conjunctiva either as a powder or as a 1 or 2 per cent. solution. The chief drawback to the use of optochin, however, is its toxic action on the retina, and a number of cases of temporary blindness in man have been recorded from its employment. Complete blindness may continue for some days, while normal vision may not be recovered for at least two months after cessation of administration of the drug.

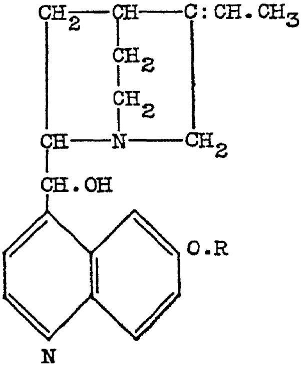
A number of cupreine derivatives have been prepared by Butler and his colleagues (1935).

Certain of these compounds, more especially ethyldihydrocupreine, and *apo*quinine (*apo*cupreine) derivatives, such as ethyl*apo*cupreine, *iso*propyl*apo*cupreine, have been found by Dawson *et al.* (1937) to produce damage in the ganglionic layer of the retina of dogs. Quinine, which, like the other cinchona alkaloids, has a vasoconstrictor action, also damages the retina, but hydroxyethyl*apo*cupreine appears to have no toxic effect on the retinal cells.

Experimental studies on *apo*cupreine, ethyl*apo*cupreine and hydroxyethyl*apo*cupreine have been carried out by Maclachlan, Permar, Johnston and Kenney (1934), and Johnston, Burchell, Permar and Maclachlan (1937).



Ethyldihydrocupreine



apo*Quinine* (apo*Cupreine*) *Derivatives*

*apo*Cupreine, R = H ;
Ethyl*apo*cupreine, R = $CH_2.CH_3$;
Hydroxyethyl*apo*cupreine, R = $CH_2.CH_2.OH$.

The results obtained may be summarised as follows : For mice the most toxic preparation is ethyl*apo*cupreine, followed by optochin, hydroxyethyl*apo*cupreine and *apo*cupreine. Even with the most toxic, ethyl*apo*cupreine, serum concentration levels which kill pneumococci *in vitro* could be reached *in vivo* without approaching the limit of safety for life. Ethyl*apo*cupreine was the most active in protecting mice inoculated intraperitoneally with highly virulent pneumococci followed by optochin and hydroxyethyl*apo*cupreine. However, in man, concentrations of hydroxyethyl*apo*cupreine in the blood sufficient to kill pneumococci could be readily obtained by the administration by mouth of doses well within the margin of safety. Although *in vitro apo*cupreine had a killing action on pneumonia, *in vivo* it was not possible by the oral administration of doses equal to those of ethyl*apo*cupreine and hydroxyethyl*apo*cupreine to endow the sera of patients with any pneumococcicidal power.

The action of ethyl*apo*cupreine and hydroxyethyl*apo*cupreine in man in pneumonia and pneumococcal empyema has been studied by Maclachlan *et al.* (1937). Ethyl*apo*cupreine was given to twenty-four patients with pneumonia in daily doses of from 40 to 60 grains per day for three or four days. Ten patients recovered, but five of the twenty-four developed total blindness which lasted for some days. Hydroxyethyl*apo*cupreine, either in the form of the dihydrochloride or preferably the monohydrochloride, was administered to 200 patients without causing any visual disturbances though occasionally some buzzing in the ears was noted. At first only 60 grains (3·88 gm.) a day were given : latterly, however, 15 grains (0·97 gm.) were given by mouth every three

Period	Mortality			
	Treated cases	Deaths	Untreated cases	Deaths
1935–1936 .	46	17 (37%)	48	20 (42%)
1936–1937 .	100	27 (27%)	46	29 (63%)
1935–1937 .	146	44 (30%)	94	49 (52%)

hours day and night, with a total of 400 to 900 grains (25·9 to 58·2 gm.) a week. Intravenous injections of 15 grains dissolved in from 50 to 75 c.c. of distilled water have also been given in acute cases. The results in 146 cases of lobar pneumonia were as shown in table on p. 472.

Pneumococcal empyema in children was treated with optochin by Gralka (1922) with apparent success, while Maclachlan *et al.* (1937) also claim good results by the injection into the pleural cavity of hydroxyethyl*apo*cupreine in the form of a 1 or 2 per cent. solution in distilled water.

References

Butler, C. L., Nelson, W. L., Renfrew, A. G., and Cretcher, L. H., 1935, Cinchona alkaloids in pneumonia. I. Miscellaneous alkaloids and some hydrocupreine ethers. *J. Amer. chem. Soc.*, **LVII.**, 738.

Butler, C. L., Renfrew, and Cretcher, L. H., 1935, Cinchona alkaloids in pneumonia. III. *apo*Cupreines (*apo*Quinines). *Ibid.*, **LVII.**, 1083.

Cohen, S. S., 1919, Present status of the definite treatment of the pneumonias: objects, indications, and methods of use of quinine, pituitary principle and digitalis. *J. Amer. med. Ass.*, **LXXIII.**, 1741.

Dawson, W. T., Permar, H. H., Johnston, J. M., and Maclachan, W. W. G., 1937, An experimental study of the variations in the production of visual disturbances by certain new cinchona derivatives. *Amer. J. med. Sci.*, **CXCIII.**, 543.

Gralka, R., 1922, Ueber die Behandlung von Pneumokokkenempyemen im Kindesalter mit Optochin. *Mschr. Kinderheilk.*, **XXIII.**, 280.

Johnston, J. M., Burchell, H. B., Permar, H. H., and Maclachlan, W. W. G., 1937, Further experiments on the effect of certain quinine derivatives on the Pneumococcus. *J. Pharmacol.*, **LXI.**, 364.

Kemkes, B., 1937, Zur Chemotherapie der Pneumokokkeninfektion. *Klin. Wschr.*, **XVI.**, 1041.

Maclachlan, W. W. G., Permar, H. H., Johnston, J. M., and Burchell, H. B., 1937, Clinical observations on the treatment of pneumonia and empyema by some quinine derivatives, especially, hydroxyethyl*apo*-cupreine. *Amer. J. med. Sci.*, **CXCIV.**, 474.

Maclachlan, W. W. G., Permar, H. H., Johnston, J. M., and Kenney, J. R., 1934, Some effects of quinin derivatives in experimental pneumococcus studies. *Ibid.*, **CLXXXVIII.**, 699.

Moore, H. F., and Chesney, A. M., 1918, A further study of ethylhydrocupreine (optochin) in the treatment of acute lobar pneumonia. *Arch. intern. Med.*, **XXI.**, 659.

Morgenroth, J., and Bieling, R., 1917, A further study of ethylhydrocuprein (optochin) in the treatment of acute lobar pneumonia. *Arch. intern. Med.*, **XXI.**, 659.

Morgenroth, J., and Levy, R., 1911, Chemotherapie der Pneumokokken infektion. *Berl. klin. Wschr.*, **XLVIII.**, 1560, 1979.

Renfrew, A. G., and Cretcher, L. H., 1935, Cinchona alkaloids in pneumonia. II. Ketone formation with sodium amide. *J. Amer. chem. Soc.*, **LVII.**, 738.

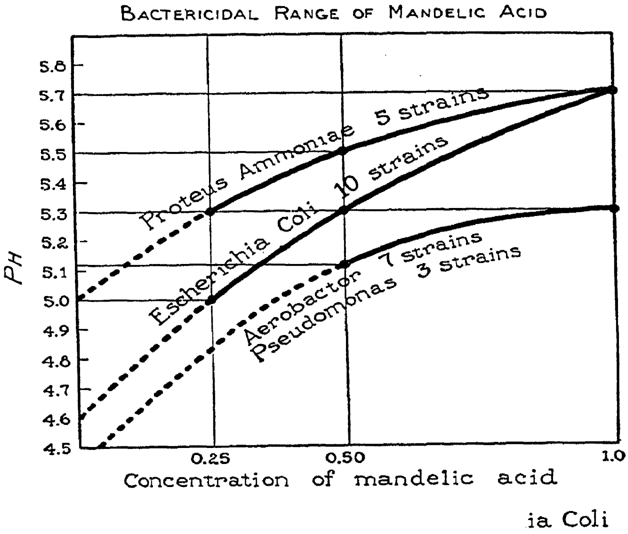
MANDELIC ACID

Although the administration of drugs such as acriflavine and hexylresorcinol has in certain cases had a curative action on bacillary infections of the urinary tract, the most interesting results have been obtained by the use of mandelic acid.

In 1917, Shohl and Janney found that the growth of bacteria in urine could be influenced by the hydrogen ion concentration, the growth of *Bacterium coli*, in particular, being retarded in a urine of *p*H 5 or less, or on the alkaline side of *p*H 9·2. Attempts were, therefore, made to keep the urine acid by administering ammonium chloride, followed, either intravenously or orally, by hexamine. Later Clark (1931) and Helmholz (1931) suggested that the use of the ketogenic diet, containing large amounts of fat and an extremely small amount of carbohydrate, would bring about the same results as regards the acidification of the urine. It was soon found, however, that acidity of the urine was not the only factor inhibiting the growth of *Bact. coli*, but that in all probability some bactericidal substance was actually present in the urine. Fuller (1933) demonstrated this bactericidal substance, for after removal of urea, inorganic salts, phosphatides, proteoses, bases, amino-acids and lactic acid, the bacteriostatic activity of the urine still remained. The lævorotatory form of β-hydroxybutyric acid was also still, however, present and to this substance was due the restraining action on the growth of the colon bacillus. As the ketogenic diet is by no means easy for many persons to take, owing to the strict regimen and the nauseating ingredients, and the amount of β-hydroxybutyric acid in the urine is not large, Fuller suggested giving the acid by mouth. Investigations quickly showed, however, that given by mouth it was quite ineffective. This result was not surprising, for if the body is in a normal metabolic state the utilisation of the usual amount of ingested carbohydrate is quite sufficient to oxidise completely the β-hydroxybutyric acid before it can be excreted by the kidneys. It was shown, however, by Cook and Braasch (1935), and Helmholz and Osterberg (1935 and 1936[1]), that the introduction of β-hydroxybutyric into urine kept at *p*H 4 rapidly inhibits the growth of a number of micro-organisms.

The next advance was made by Rosenheim (1935), who attempted to find some keto or hydroxy acid which would combine resistance to oxidation in the body with effective bacteriostatic power and low toxicity. In 1883, Shotten had found that mandelic acid, on administration by mouth to the dog, was excreted unaltered in the urine. This result in the dog was confirmed by Rosenheim, while large doses were found to be non-toxic. Preliminary observations on man showed that a beneficial action was exerted on infections with *Bact. coli.* Mandelic acid, or phenyl-glycollic acid, α-hydroxy-α-toluic acid ($C_6H_5.CH(OH)COOH$), is a synthetically prepared racemic (*d*-*l*) compound. It is a white crystalline substance, melting at 118°–120° C., and giving a colourless, clear solution in water, alcohol and alkali. The good results obtained by Rosenheim were confirmed by Lyon and Dunlop (1935) and Holling and Platt (1936), who suggested the use of mandelic acid salts, more especially ammonium mandelate. Previously the use of mandelic acid, as such, had necessitated the administration of ammonium chloride to render the urine sufficiently acid. Thus Carroll, Lewis and Kappel (1936) placed their patients on an acid-ash diet and gave 1 gm. of ammonium chloride four times daily. The acidity of the urine was usually at a *p*H 5·5 at the end of twenty-four hours ; mandelic acid was then prescribed in the following formula : mandelic acid 48 gm., sodium bicarbonate 25·6 gm., distilled water 480 gm., with the addition of a suitable flavouring agent.

In some cases nausea, diarrhœa, dysuria, or even hæmaturia, occurred, but this was probably due to the ammonium chloride rather than to the mandelic acid, for the substitution of sodium diphosphate for ammonium chloride relieved these symptoms. A 10 per cent. solution of ammonium mandelate in most cases renders the urine acid without the addition of an acidifying drug. However, it must be remembered that both sodium and ammonium mandelate are very hydroscopic and decompose readily, while mandelic acid is quite stable. Cook and Buchtel (1936) have suggested the use of ethanolamine mandelate, which also eliminates the use of an acidifying drug. Schnohr (1937) used calcium mandelate with success: in the stomach it is broken down to mandelic acid and calcium chloride, and in eight cases the *p*H of the urine rapidly became from 4·8 to 5·5. Hryntschak (1937) and Melton and Rosenheim (1938) also used calcium mandelate, which is quite tasteless. Although insoluble in water, it can be given mixed with milk or food, but should not be given for more than ten days at a time, otherwise nausea and acute dislike of the drug



Rate of excretion and bactericidal power of mandelic acid in the urine (Helmholz and Osterberg, 1936²).

may appear. For adults 3·5 gm. are administered four times a day, while children above the age of six received two-thirds of the adult dose: fluid intake must be limited to two pints a day.

Gram (1938) has carefully compared the results of treatment by calcium mandelate with those following treatment by salol and calcium chloride, on the one hand, and hexamine and ammonium chloride on the other. Calcium mandelate gave much the best results and could be used without risk in pregnancy.

In the majority of urinary infections the organisms are bacillary in type, and in large measure are *Bact. coli*, *Aerobacter*, *Proteus* and *Pseudomonas*. Helmholz and Osterberg (1936 [1]) have studied the relationship between the *p*H and the concentration of mandelic acid in the urine. All strains of *Bact. coli* were uniformly killed at a certain *p*H and concentration of acid. Certain strains of *Aerobacter* and *Pseudomonas* are killed under the same conditions, but the greater number of strains are killed only by a higher concentration of acid or a lower *p*H or both. The organism most readily killed is *Proteus vulgaris*, but the difficulty in the treatment of infections with *Proteus* is the inability to keep acid the urine because of the alkalinity produced by the growth of *Proteus* in the urine. Staphylococci appear to be as readily killed off as bacillary organisms.

In vivo the most satisfactory results have been obtained in infections due to *Bact. coli*, although in certain cases there is a tendency for organisms to reappear in the urine. This tendency to relapse is perhaps not surprising when the original focus is not situated directly in the urinary tract, but merely drains into it. Carroll, Lewis and Kappel (1936), however, obtained sterile urines in 81 per cent. of their cases, the bacteria disappearing in from four to twelve days. In only one of six cases of *Proteus* infection did the urine become sterile. Dolan (1936) failed to cure three cases of staphylococcus infection, while a case of mixed infection due to staphylococci, diplobacilli and possibly tubercle bacilli became worse. Carroll and his co-workers (1936) obtained sterile urine in only one of six cases of staphylococci infection. One case of infection due to *Streptococcus viridans* was slightly improved, while two cases of infection with *Streptococcus fœcalis* were cured (Helmholz and Osterberg, 1936 [1]). Summerfeldt and Brown (1938) reported good results in 52 cases, two of which were due to *Staphylococcus aureus* and one to *Bact. dysenteriœ* Flexner. Of 20 cases observed for periods up to sixteen months, pyuria recurred in four instances and bacilluria in three. The other 13 cases remained well. Sodium or ammonium mandelate was the compound employed. Reimann (1938) successfully treated a case of typhoid bacilluria. Apart from diarrhœa, nausea and vomiting, the only serious complication is that reported by

Dolan (1936), who noted the occasional occurrence of hæmaturia.

Helmholz and Osterberg (1936 [2 & 3]) have already shown in studies on animals that excessive doses of mandelic acid will destroy the renal function and may even cause death. Cook and Buchtel (1936) found in a series of normal persons, who were given 12 gm. of mandelic acid daily for a week, that in all cases the blood urea was increased and the percentage of phenolsulphonphthalein excreted decreased. Whether or not this apparent decrease in renal function was due to lowered fluid intake or to the toxic action of the drug on the kidney is at present uncertain, but caution should certainly be used in prescribing mandelic acid for the aged and for those with a reduced renal function, and in any case should not be given for more than ten to fourteen days at a time.

Helmholz (1937) has pointed out that mandelic acid is dependent for its action on a concentration greater than 0·5 per cent., so that it is not likely to work unless kidney function is normal or nearly so. Mandelic acid differs from sulphanilamide in being ineffective in *Proteus* infections, where it is difficult to render the urine alkaline, but is highly effective in infections due to *Streptococcus fœcalis* in which sulphanilamide is almost useless.

References

CARROLL, G., LEWIS, B., and KAPPEL, L., 1936, Mandelic acid as a urinary antiseptic. *J. Amer. med. Ass.*, **CVII.**, 1796.

CLARK, A. L., 1931, *Escherichia coli* bacilluria under ketogenic treatment. *Proc. Staff Meet. Mayo Clin.*, **VI.**, 605.

COOK, E. N., and BRAASCH, W. F., 1935, Further studies on the use of ketogenic diet for bacilluria. *J. Urol.*, **XXXIII.**, 583.

COOK, E. N., and BUCHTEL, H. A., 1936, Mandelic acid in the treatment of infections of the urinary tract. *J. Amer. med. Ass.*, **CVII.**, 1799.

DOLAN, L. P., 1936, Experiences with ammonium mandelate in urinary infections : a report of results obtained in sixteen cases of various types of infections regardless of the existing pathologic condition. *Ibid.*, **CVII.**, 1800.

FULLER, A. T., 1933, The ketogenic diet : nature of the bactericidal agent. *Lancet*, *i.*, 855.

GRAM, H. C., 1938, Calcium mandelate treatment of pyelitis compared with other therapeutic measures. *Acta med. Scand.*, **XCIV.**, 615.

HELMHOLZ, H. F., 1931, The ketogenic diet in the treatment of pyuria of children with anomalies of the urinary tract. *Proc. Staff Meet. Mayo Clin.*, **VI.**, 609.

HELMHOLZ, H. F., 1937, A comparison of mandelic acid and sulfanilamide as urinary antiseptics. *J. Amer. med. Ass.*, **CIX.**, 1039.

HELMHOLZ, H. F., and OSTERBERG, A. E., 1935, Bactericidal effect in urine of levorotatory and racemic *beta*-oxybutyric acid. *Proc. Staff Meet., Mayo Clinic*, X., 620.

HELMHOLZ, H. F., and OSTERBERG, A. E., 1936[1], The bactericidal effect of lævorotatory and racemic *beta*-oxybutyric acid in urine. *J. Urol.*, XXXV., 86.

HELMHOLZ, H. F., and OSTERBERG, A. E., 1936[2], The rate of excretion and bactericidal power of mandelic acid in the urine. *Proc. Staff Meet. Mayo Clin.*, XI., 373.

HELMHOLZ, H. F., and OSTERBERG, A. E., 1936[3], Rate of excretion and bactericidal power of mandelic acid in the urine. *J. Amer. med. Ass.*, CVII., 1794.

HOLLING, H. E., and PLATT, R., 1936, Mandelic acid and ammonium mandelate in the treatment of urinary infections. *Lancet*, *i.*, 769.

HRYNTSCHAK, T., 1937, Über die Mandelsäurebehandlung der Harninfektion. *Med. Klin.*, XXXIII., 1099.

LYON, D. M., and DUNLOP, D. M., 1935, Mandelic acid in the treatment of urinary infections. *Brit. med. J.*, *ii.*, 1096.

MELTON, G., and ROSENHEIM, M. L., 1938, Calcium mandelate. *Lancet*, *i.*, 494.

REIMANN, H. A., 1938, Typhoid pyelonephritis, renal typhoid fever. *J. Amer. med. Ass.*, CXI., 691.

ROSENHEIM, M. L., 1935, Mandelic acid in the treatment of urinary infections. *Ibid.*, *i.*, 1032.

SCHNOHR, E., 1937, Treatment of urinary infections with calcium mandelate. *Ibid.*, *i.*, 1104.

SHOHL, A. T., and JANNEY, J. H., 1917, The growth of *Bacillus coli* in urine at varying hydrogen ion concentrations. *J. Urol.*, *i.*, 211.

SHOTTEN, C., 1883, Ueber die Quelle der Hippursäure im Harn. *Z. physiol. Chem.*, VIII., 60.

SUMMERFELDT, P., and BROWN, A., 1938, Mandelic acid in treatment of pyuria. *Canad. med. Ass. J.*, XXXVIII., 352.

MERCURY COMPOUNDS

A considerable number of organic mercurial compounds have now been tested for their action both in septicæmia and on localised bacterial lesions. The most important of these compounds are mercurochrome, metaphen, mercurophen, merthiolate and phenylmercuric nitrate.

Mercurochrome-220 soluble, disodium dibromohydroxymercurifluorescein, $C_{20}H_7O_5Br_2HgOHNa_2$, was first prepared by White (1920). Metaphen is, according to Raiziss and Severac (1927), 4-nitro-3 : 5-bisacetoxy-mercuri-2-cresol ; according to N. N. R., however, it is 4-nitro-5-hydroxymercuri-2-cresol. Mercurophen is sodium hydroxymercuri-*ortho*-nitrophenolate. It was first employed by Schamberg, Kolmer and Raiziss (1919), Kolmer and

Raiziss (1919) and others, who demonstrated its unusual antiseptic and disinfectant properties.

Merthiolate is sodium ethyl-mercurithiosalicylate, while phenylmercuric nitrate, prepared by Otto in 1870, has been suggested as a skin disinfectant and urinary antiseptic by Birkhaug (1933), and Weed and Ecker (1931). Pyman and Stevenson (1934), however, point out that only the basic salt, $C_6H_5HgOH.C_6H_5.HgNO_3$, has been employed.

Mercurochrome has unfortunately failed to fulfil the hopes which were at first entertained of it as an antidote in septicæmia. That it is by no means a perfect germicide was pointed out by Buchsbaum and Bloom (1931) and Salle and Lazarus (1935) who, using slightly different techniques, compared the highest dilutions capable of killing *Staphylococcus aureus* and inhibiting the growth of tissue cultures.

Toxicity indexes obtained by Salle and Lazarus were for metaphen 12·7, phenol 12·9, merthiolate 35·3, and mercurochrome 262, the smaller the toxicity index the more efficient being the germicide.

Birkhaug (1930) also obtained results *in vitro* which seemed to suggest that metaphen was a more efficient germicide than mercurochrome, as shown by the following figures :—

Test organism	Highest dilution of metaphen inhibiting growth.	Highest dilution of mercurochrome inhibiting growth
Staphylococcus aureus .	1 in 16,400,000	1 in 18,000
Streptococcus hæmolyticus .	1 in 6,400,000	1 in 32,000
Gonococcus . . .	1 in 1,600,000	1 in 36,000
Bacterium coli . . .	1 in 200,000	1 in 32,000

Observations *in vivo* by Douglas and Birkhaug (1933 [1, 2]) failed to confirm these findings for administration of metaphen in artificial septicæmias in mice, rabbits and rats induced by *Pneumococcus* Type I and *Streptococcus hæmolyticus* produced no benefit, but rather accelerated a fatal issue, usually as the result of tubular nephritis.

While streptococci, smooth variety, suspended in broth are killed by metaphen in a dilution of 1 in 130,000,000, when suspended in defibrinated blood they are not killed by a dilution of 1 in 4,000.

An explanation of the failure of mercurial antiseptics injected *in vivo* has been given by Smith, Czarnetzky and Mudd (1936), who have pointed out that antiseptic action is almost wholly due to their mercury content. When a litre of serum is mixed with excess of a mercurial such as metaphen or mercurochrome it is found that from 0·29 to 0·39 gm. of mercury is irreversibly bound by the serum proteins. The non-polar compounds of serum and mercury are completely devoid of bactericidal action ; any killing action still possessed by the mixture is due to unbound mercury present in excess.

If it is desired to sterilise the blood-stream by mercurials such as mercurochrome or metaphen, many times the lethal dose must be administered. Barthelme (1938), however, claims to have obtained excellent results by the intravenous injection of 10 c.c. of 1 in 1,000 metaphen in the treatment of tularemia, a disease in which sulphonamide compounds have proved useless.

Emmett (1938) believes that intravenous injections of from 5 to 10 c.c. of a 1 per cent. solution of mercurochrome in cases of acute pyelonephritis is followed by a rapid fall of temperature and the termination of the acute phase of the disease.

As disinfectants for internal use, organic mercurials have thus proved unsatisfactory, although as antiseptics for such purposes as cleansing the skin before operation they possibly possess some value.

References

BARTHELME, F. L., 1938, Metaphen intravenously in the treatment of tularemia. *J. Chemotherap.*, **XIV.**, 117.

BIRKHAUG, K. E., 1930, Metaphen (4-nitro-3 : 5-bisacetoxymercuri-2-cresol). I. A comparative study of commonly used disinfectants and antiseptics. I. Histologic changes produced by the intravenous administration of metaphen in rabbits. *J. Amer. med. Ass.*, **XCV.**, 917.

BIRKHAUG, K. E., 1933, Phenyl-mercuric-nitrate. *J. inf. Dis.*, **LIII.**, 250.

BUCHSBAUM, R., and BLOOM, W., 1931, Relative toxicity of antiseptics on bacteria and tissues in cultures. *Proc. Soc. exp. Biol., N.Y.*, **XXVIII.**, 1060.

DOUGLAS, R. G., and BIRKHAUG, K. E., 1933^1, Metaphen. I. Results of the use of metaphen in experimental septicæmia. *J. inf. Dis.*, **LIII.**, 55.

DOUGLAS, R. G., and BIRKHAUG, K. E., 1933 ^{2}, Metaphen. II. Its experimental evaluation as an antiseptic and disinfectant. *Ibid.*, **LIII.**, 71.

EMMETT, J. L., 1938, Antipyretic action of intravenous administration of mercurochrome in acute pyelonephritis. *J. Urol.*, **XL.**, 269.

KOLMER, J. A., and RAIZISS, G. W., 1919, Sodium oxy-mercury-*ortho*-nitrophenolate (mercurophen). *Ibid.*, **XXIV.**, 547.

OTTO, R., 1870, Ueber die Darstellung von Acetonen mittelst Quecksilberdiphenyl. *J. prakt. Chem.*, **I.**, 179.

PYMAN, F. L., and STEVENSON, H. A., 1934, Phenylmercuric nitrate. *Pharm. J.*, **CXXXIII.**, 269.

RAIZISS, G. W., and SEVERAC, M., 1927, Metaphen (4-nitro-3, 5-bisacetoxymercuri-2-cresol) and its bactericidal properties. *J. inf. Dis.*, **XL.**, 446.

SALLE, A. J., and LAZARUS, A. S., 1935, A comparison of the resistance of bacteria and embryonic tissue to germicidal substances. I. Merthiolate. II. Metaphen. III. Mercurochome. *Proc. Soc. exp. Biol., N.Y.*, **XXXII.**, 665, 937 and 1,057.

SCHAMBERG, J. F., KOLMER, J. A., and RAIZISS, G. W., 1919, The chemotherapy of mercurial compounds. *Tr. Coll. Phys. Phila.*, **XXXIX.**, 33.

SMITH, D. E., CZARNETZKY, E. J., and MUDD, S., 1936, The mechanism of inactivation of mercurial antiseptics by serum and its implications regarding the possibility of intravenous antisepsis. *Amer. J. med. Sc.*, **CCXII.**, 790.

WEED, L. A., and ECKER, E. E., 1931, The utility of phenyl-mercury-nitrate as a disinfectant. *J. inf. Dis.*, **XLIX.**, 440.

WHITE, E. C., 1920, Mercury derivatives of phthaleins. *J. Amer. chem. Soc.*, **XLII.**, 3355.

MISCELLANEOUS COMPOUNDS

In view of the successful use of sulphonamides of the aromatic series in the treatment of various bacterial diseases, chemists have naturally suggested the trial of allied organic compounds containing sulphur in different stages of oxidation. Among the many compounds made and tested on haemolytic streptococci by Gray and Buttle and their collaborators (Buttle *et al.* (1938); *cf.* also Gley, 1937) are the following :—

(*a*) *Sulphinic Acids.* *p*-Acetylaminobenzenesulphinic acid, as found by Gley (1937) and Buttle (1937), is active, whereas the corresponding sulphonic acid is not. 2-Pyrrolidone-5-carboxyaminobenzene-4′-sulphinic acid, prepared originally by Gray, is as active as sulphanilamide and is less toxic, since 100 mgm. by mouth does not cause any symptoms.

(*b*) *Diphenyl Derivatives.* Benzidine-*o*-sulphone is inactive.

(*c*) Trithioaniline, sulphamide and aminosulphonic acid are also inactive.

Among non-sulphur-containing compounds, Levaditi, Girard and Vaisman (1938) found that hydroquinone, its acetyl derivative and 4 : 4′-dihydroxyazobenzene, were active in gonococcal and meningococcal infections in the mouse as well as against the endotoxins of gonococcus. The effect on streptococcal infections in the mouse of Sdt. 386 B has been studied by Domagk (1935). The composition of the compound has not been disclosed, except that it is said to contain 18 per cent. of arsenic and 20 per cent. of antimony. In streptococcal infections of the mouse it was found to possess a chemotherapeutic index of 1 : 4 or 1 : 5, but its chief activity is against *Bartonella* infections of rats, where it has a chemotherapeutic index of 1 in 3,500 (Kikuth, 1934).

A considerable number of substances have been found to inhibit the growth of fungi *in vitro*, such as the series of alkyl dimethylbenzyl ammonium chlorides tested by Dunn (1938) on *Actinomyces gedanensis* and *Epidermophyton inguinale.* Ch'in, Kurotchin and Mu (1938) have found that when tested on *Actinomyces bovis,* neoarsphenamine, silver arsphenamine and sulpharsphenamine are fungicidal both *in vitro* and to a small extent *in vivo.*

References

Buttle, G. A. H., 1937, Discussion on the treatment of bacterial diseases with substances related to sulphanilamide. *Proc. R. Soc. Med.*, XXXI., 154.

Buttle, G. A. H., and Dewing, G. T., Foster, G. E., Gray, W. H., Smith, S., and Stephenson, D., 1938, The action of substances allied to 4 : 4-diaminodiphenylsulphone in streptococcal and other infections in mice. *Biochem. J.*, XXXII., 1101.

Ch'in, T. L., Kurotchin, T. J., and Mu, J. W., 1938, The fungistatic and fungicidal actions of arsenical compounds *in vitro* and *in vivo*. *Chin. med. J.* (Supplement II), 325.

Domagk, G., 1935, Chemotherapie der bakteriellen Infektionen. *Z. angew. Chem.*, XLVIII., 657.

Dunn, C. G., 1938, Fungicidal properties of alkyl-dimethyl-benzyl-ammonium chlorides. *Proc. Soc. exp. Biol., N.Y.*, XXXVII., 661.

Gley, P., 1937, Action antistreptococcique de certains dérivés sulfurés organiques. *C. R. Acad. Sci., Paris*, CCIV., 1907.

Kikuth, W., 1934, The Bartonella and related parasites in man and animals (Oroya fever and Verruga peruviana). *Proc. R. Soc. Med.*, XXVII., 1241, 1.

Levaditi, C., Girard, A., and Vaisman, A., 1938, Action curative et anti-endotoxique de certains dérivés benzéniques non soufrés dans la gonococcie et la meningococcie expérimentales. *C. R. Soc. Biol., Paris*, CXXVIII., 305.

ALLANTOIN AND COD LIVER OIL

Two other substances which have been used in the treatment of chronic wound infections, allantoin and cod liver oil, require brief mention, for although there is no evidence that either of these substances is directly bactericidal, there is evidence that they accelerate wound healing.

Allantoin is not a new remedy, for Macalister (1912) noted that comfrey root derived from *Symphytum officinale* had long been used as a remedy in folk medicine in Great Britain, and comfrey root contains from 0·6 to 0·8 per cent. of allantoin. It had also long been known that wounds infested with maggots tended to heal rapidly. Baer (1930 and 1931) introduced the use of specially bred maggots, while Robinson (1935[1, 2]) found that allantoin, which is excreted by maggots, is almost as efficient in promoting wound healing. Later Robinson (1936) believed that urea was the essential substance derived from maggots that stimulated the healing of wounds. Urea had, however, been employed more than twenty years before as a bactericide in the treatment of wounds by Symmers and Kirk (1915). Further investigation is required of the mechanism underlying the supposed action on chronic suppuration of maggots, allantoin and urea. Although allantoin is supposed to be capable of stimulating the growth of tissue cells, experimental results have not been conclusive. Shipp and Hetherington (1936) found that allantoin exerted a slight but by no means insignificant stimulation on the growth of chicken fibroblasts in tissue culture, while Chu (1938) found that pure cultures of chicken epithelial cells and chicken fibroblasts showed a retardation of growth which was in proportion to the concentration of allantoin present.

Cod liver oil has also been applied as a dressing for wounds, and more especially in osteomyelitis, where it appears to have an accelerating action on healing (*cf.* Chatterjee, 1937). In diabetics, Brandaleone (1938) has recorded very rapid improvement in the healing of ulcers of the feet. The active component of the oil is still uncertain (*cf.* Löhr and Unger, 1937). Crude cod liver oil, according to Daver (1935), appears to be more satisfactory than purified cod liver oil.

References

Baer, W. S., 1930, Sacro-iliac joint ; arthritis deformans ; viable antiseptic in chronic osteomyelitis. *Proc. int. Assemb. post-grad. Ass. N. Amer.*, **V.**, 371.

Baer, W. S., 1931, The treatment of chronic osteomyelitis with the maggot (larva of the blow fly). *J. Bone Jt. Surg.*, **XIII.**, 438.

Brandaleone, H., 1938, Effect of direct application of cod liver oil on healing of ulcers of the feet in patients with diabetes mellitus. *Ann. Surg.*, **CVIII.**, 141.

Chatterjee, B., 1937, The use of cod liver oil in infected wounds. *Indian med. Gaz.*, **LXXII.**, 721.

Chu, H. J., 1938, Influence of allantoin on growth of chicken epithelial cells and chondroblasts in culture. *Proc. Soc. exp. Biol., N.Y.*, **XXXVIII.**, 99.

Daver, M. B., 1935, Crude cod liver oil in treatment of wounds. *Brit. med. J.*, *i.*, 169.

Löhr, W., and Unger, F., 1937, Tierexperimentelle Untersuchungen über die Wundheilung mit Lebertran und die Wirkung seiner Bestandteile. *Arch. klin. Chir.*, **CLXXXIX.**, 321.

Macalister, C., 1912, A new cell proliferant : its clinical application in the treatment of ulcers. *Boston med. surg. J.*, **I.**, 10.

Robinson, W., 1935 [1], Stimulation of healing in non-healing wounds by allantoin occurring in maggot secretions and of wide biological distribution. *J. Bone Jt. Surg.*, **XVII.**, 267.

Robinson, W., 1935 [2], Allantoin, a constituent of maggot excretions, stimulates healing of chronic discharging wounds. *J. Parasit.*, **XXI.**, 354.

Robinson, W., 1936, Use of urea to stimulate healing in chronic purulent wounds. *Amer. J. Surg.*, **XXXIII.**, 192.

Shipp, M. E., and Hetherington, D. C., 1936, Effect of allantoin upon fibroblasts from cardiac explants in tissue culture. *Proc. Soc. exp. Biol., N.Y.*, **XXXV.**, 180.

Symmers, W. S. C., and Kirk, T. S., 1915, Urea as a bactericide and its application in the treatment of wounds. *Lancet*, *ii.*, 1237.

CHAPTER X

THE CHEMOTHERAPY OF VIRUS INFECTIONS

ALTHOUGH the chemotherapy of virus infections has as yet made but little headway, the discovery of the sulphonamide group of drugs has nevertheless raised hopes that a successful chemotherapy of these infections is by no means impossible.

It would, however, be of little interest to mention the large number of medicaments that have entirely failed to exert any chemotherapeutic action on the course of virus infections. These failures, it has been suggested, are largely due to the fact that the viruses are obligatory intracellular parasites, but in view of the treatment of meningococcal and gonococcal infections with sulphonamide derivatives this objection is of little weight. A more plausible explanation would seem to be, that owing to their high degree of parasitism, the majority of viruses have become so specialised as to require for their nourishment only a few chemical substances which are found fully prepared for them within the cell. To all other substances they are inert. If this suggestion be correct, the larger viruses, some of which pass through a definite life cycle, should, owing to their greater complexity, probably prove more amenable to chemotherapeutic action.

With the discovery of the action of the sulphonamide group of drugs in bacterial infections it was, of course, only natural that investigations should be made of the effect of these drugs in virus diseases. The action of these compounds has been tested in the following diseases with, up to the present, negative results :—

Anterior poliomyelitis in the rhesus monkey (Kelson, 1937 ; McKinley, Acree and Meck, 1937 ; Rhodes and van Rooyen, 1938) ; St. Louis encephalitis in mice (Rosenthal, Wooley and Bauer, 1937 ; Levaditi, 1938 [1]) ; herpes (Levaditi and Vaisman, 1935) ; virus myxomatosum in rabbits (McKinley *et al.*, 1937 ;

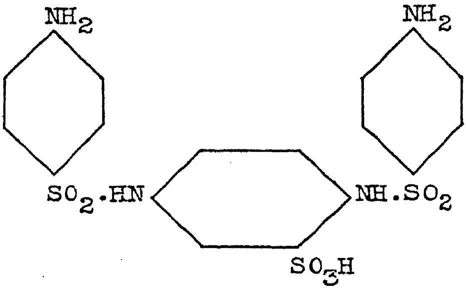
Rhodes and van Rooyen, 1938) ; Shope rabbit fibroma (McKinley *et al.*, 1937) ; feline distemper (Whicher and Smith, 1937) ; yellow fever in the rhesus monkey and mouse (Findlay and MacCallum, 1938) ; and in man (Alexander, 1938) ; Rift Valley fever in the mouse (Findlay and MacCallum, 1938).

In contradistinction to these entirely negative results, positive effects have been claimed in the following virus infections :—

The common cold, lymphocytic choriomeningitis, canine distemper, influenza and lymphogranuloma inguinale. In the common cold Elyan (1938) claimed that sulphanilamide is capable of aborting the disease. Others, however, have seen colds developing during the course of the sulphanilamide treatment of other diseases. In lymphocytic choriomeningitis in mice, Rosenthal, Wooley and Bauer (1937) reported that they had obtained protection with prontosil given subcutaneously in oil, provided that the drug was given immediately after the injection of the virus, the infecting dose of virus was small, and the doses of the drug near the tolerated limit. Prontosil soluble, as well as some other related sulphonamides, not containing an azo-linkage, although more active than prontosil itself in certain bacterial infections, were inactive in lymphocytic choriomeningitis. In every experiment with prontosil itself, even when complete protection was not attained, some prolongation of life was observed in the treated mice. These results lack confirmation. Ronse (1938) and Levaditi (1938 [1]), working with a French strain of the virus of lymphocytic choriomeningitis, and Findlay and MacCallum (1938), with two English strains, failed to obtain either complete protection or even any prolongation of life with either prontosil or sulphonamide derivatives.

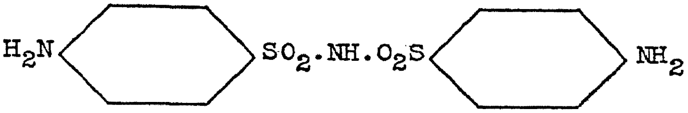
In influenzal infections in mice, Rosenthal *et al.* (1937) failed to note any curative action on the part of prontosil or sulphanilamide. Levaditi (1938 [1]), however, found that with a feebly pathogenic influenza virus 4-nitro-4′-aminodiphenylsulphoxide, given in maximum tolerated doses, produced some delay in the time of death of the mice, but when the pathogenicity of the virus was increased there was no difference in the time of death of control and treated mice. Oakley (1938) found that mice treated with *p*-aminobenzenesulphonamide and 4-benzylideneamino-4′-aminodiphenylsulphone died sooner and showed a higher mortality than did the corresponding controls. Between animals treated with 4 : 4′-diaminodiphenylsulphone or sodium sulphanilyl-sulphanilate and the controls there was no significant difference. Mice, however, treated with a glucose derivative of 4 : 4′-diaminodiphenylsulphone (*cf.* p. 377) showed no advantage over the controls when the infecting dose of virus was a large one, but if the virus inoculum contained only 10 or 100 minimal infective doses there was a small but significant difference in favour of the treated mice. Recently Crossley (1938) has claimed that treatment of mice infected with influenza virus by 2 : 5-disulphanilamidobenzenesulphonic acid has produced successful cures.

According to Climenko, Crossley and Northey (1938), in addition to 2 : 5-disulphanilamidobenzenesulphonic acid, 4 : 4′-diaminobenzenesulphonimide and its methyl and ethyl derivatives all show a protective action in mice against influenza virus, provided the infecting dose of the virus is sufficiently small to delay symptoms till the fourth day after infection. When the infecting dose of the virus is increased to the point at which the majority of the control animals show acute symptoms by the second or third day after infection, with death on the third or fourth day, the drugs are completely ineffective. (In the text of their communication Climenko *et al.* describe the compounds they employed as disulphanilamide, N′-methyl disulphanilamide and N′-ethyl-

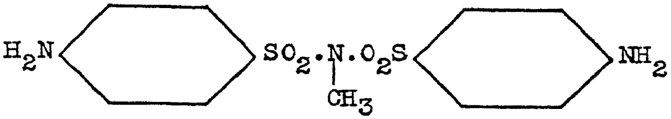


2 : 5-*Disulphanilamidobenzenesulphonic acid*

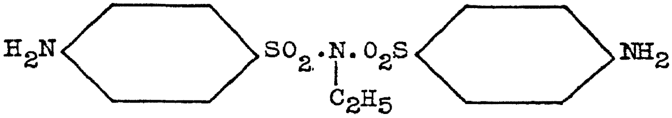
disulphanilamide: the formulæ provided do not correspond to these compounds.)



4:4′-Diaminobenzenesulphonimide



4:4′-Diaminobenzenesulphonmethylimide



4:4′-Diaminobenzenesulphonethylimide

In the treatment of naturally occurring canine distemper Daviaud and Fourneaux (1936) believed that prontosil was of some value, while Marcus and Necheles (1938) also came to the same conclusion in regard to prontosil and sulphanilamide. In the treatment of the experimental disease in dogs and ferrets, MacIntyre and Montgomerie (1938) and Dickerson and Whitney (1938) were unable to find that prontosil, sulphanilamide or sodium sulphanilyl sulphanilate had any action, these findings being in direct opposition to those of Dochez and Slanetz (1938), who claimed that sodium sulphanilyl sulphanilate exerted a curative action both in dogs and ferrets. No explanation is at present forthcoming for these divergent results. In the naturally occurring disease it is not improbable that intercurrent bacterial infection may be counteracted, apart from any possible action on the virus itself (*cf.* page 361).

Recently Levaditi (1938 [1]) has observed a retarding action by sodium 4-sulphamidobenzeneazo-3′-5′-diaminobenzoate and prontosil on the development and generalisation of pad lesions in guinea-pigs inoculated with foot and mouth disease. These results have not been confirmed.

The most striking results in the chemotherapy of virus infections have, however, been obtained in the treatment of lymphogranuloma inguinale, a disease in which, it has been claimed, antimonials such as tartar emetic or fouadin have been of value (*cf.* Schmidt and Peter, 1938). Some doubt, however, has been expressed as to whether antimony compounds have any specific action in this disease or whether their effect is not due to protein shock, since good results have also been obtained in lymphogranuloma inguinale by the injection of such substances as anti-typhoid vaccine. The first observations on the treatment of lymphogranuloma inguinale by sulphonamide compounds were made by Levaditi and Vaisman (1935), who failed to cure the meningoencephalitis produced in monkeys by the intracerebral injection of this virus by administration of prontosil. In man, however, Gjurić (1938) obtained rapid and complete cure of inguinal buboes by injections of fouadin and by the administration of prontosil by mouth. The latter drug was given for a five-day period in daily doses of 2·5 gm. followed immediately by a further administration of 2·0 gm. daily for three days; after this a period of rest was enforced for three days and the course repeated. Since fouadin injections were combined with prontosil, there is necessarily some uncertainty as to which drug was possibly responsible for the curative effect. A little later, however, Kubitzki (1938) employed prontosil by mouth and injections of prontosil soluble, without the addition of any other drug. Prompt fall in temperature and improvement of the local lesions resulted. Montel and Nguyen-Van-Tho (1938) obtained similar results. Experimentally, Levaditi (1938 [1]), in opposition to his previous results in the monkey, found that the buboes produced by lymphogranuloma inguinale virus in the groins of guinea-pigs were rapidly cured by sulphanilamide and sodium 4-sulphamidobenzeneazo-3′ : 5′-diaminobenzoate. Others, on the other hand, have found that with different strains of the virus of lymphogranuloma inguinale the buboes produced in the groins of guinea-pigs occur so irregularly and are so rapidly self-cured that it would be extremely difficult to interpret any therapeutic experiments. In infections in mice in which the virus is injected directly into the brain, Bär (1938), however, found that sulphanilamide produced a retardation in the development of symptoms, and in some cases the lives of the mice were saved; thus of 7 treated mice 3 lived, while of 6 controls only 1 survived. These results were confirmed by Levaditi (1938 [2]), and by MacCallum and Findlay (1938). Levaditi killed his mice on the eighth day after injection and found that the treated mice, as compared with controls, showed much smaller lesions, but the number of mice used was small; the most active compound was sulphanilamide. MacCallum and Findlay found that of mice given sulphanilamide subcutaneously in oil, 55 per cent. were protected against an intracerebral inoculation of a very active strain of the virus of lymphogranuloma inguinale, while only 16 per cent. of controls survived. Even more striking results were obtained by the oral administration of daily doses of 10 mgm. for a 20 gm. mouse of a glucose derivative of 4 : 4′-diaminodiphenylsulphone (*cf.* p. 377). Although of low toxicity in animals, in man this compound appears to be somewhat toxic.

Shropshear (1938) has recently treated 9 cases of rectal stricture and 1 case of chronic ulceration of the vulva with urethritis due to the virus of lymphogranuloma inguinale.

In the treatment of trachoma, Lian (1938), in the Dutch East Indies, Loe (1938), in America, and Kirk *et al.* (1938), in the Sudan, have independently found that sulphanilamide has a remarkable effect in trachoma. Although sulphanilamide by mouth may not cure every case of trachoma it rapidly improves the conjunctivitis and corneal thickening and such corneal complications as pannus, keratitis and ulcer, which show no tendency to recur. Photophobia and the continuous lachrymation were also greatly lessened. Antimony salts have also from time to time been employed with apparent success in trachoma.

These observations suggest that the chemotherapy of virus diseases does not present an impossible task, though the susceptibility of the virus of lymphogranuloma inguinale may, as previously suggested, be due to the fact that this virus has an elaborate life cycle, resembling somewhat that of psittacosis (*cf.* Findlay, Mackenzie and MacCallum, 1938). Inclusion conjunctivitis, however, which also has a developmental cycle, is unaffected by sulfanilamide (Kirk *et al.*, 1938). Apart from any action on viruses sulphonamide compounds may be of value in treating the secondary bacterial infections which complicate many virus diseases. Thus hæmorrhagic small-pox is associated with a streptococcal infection which might well be controlled by sulphanilamide, as apparently are the secondary complications of measles (*cf.* Thompson and Greenfield, 1938).

The chemotherapeutic treatment of rickettsial infections is still unsatisfactory. Kulesza (1937) in typhus fever reported 20 cures, 1 improvement, and 5 uninfluenced in a series of 26 patients treated by prontosil. Further observations are obviously required. In trachoma, which may be due to rickettsia, Selinger (1935) and Ch'i (1936) reported improvement after the local application of quinine. In Switzerland, Contat (1938) has claimed to have obtained good results in poliomyelitis by the oral administration of potassium chlorate: in addition, 5 drops of a 2 per cent. solution were instilled intranasally. Mauer (1938) has found that about 50 per cent. of mice infected with psittacosis can be saved by treatment with acriflavine.

CHEMO-PROPHYLAXIS OF VIRUS INFECTIONS

In view of the difficulties of specific treatment in virus infections, efforts have been made to elaborate measures of chemotherapeutic prophylaxis. Since the nasal mucosa is the portal of entry of many viruses the possibility has been explored of treating the nasal mucosa with chemicals so that its permeability to viruses is decreased. Armstrong and Harrison (1935) first showed that alum, sprayed up the nostrils, would protect rhesus monkeys against the intranasal instillation of poliomyelitis virus. Later, Schultz and Gebhardt (1936) and Sabin, Olitsky and Cox (1936) found that picric and tannic acids produced the same results in monkeys, while Schultz and Gebhardt (1938 [1]) found that the most effective substance in protecting monkeys was zinc sulphate. Unfortunately the practical application of these experimental findings has not been attended with great success. Armstrong (1937), using a picric acid-alum nasal spray in Alabama in 1936, observed no significant difference in the incidence of poliomyelitis between treated and untreated children, while more recently the use of zinc sulphate sprays in Canada has failed to yield results of any statistical significance (Tisdall *et al.*, 1937). The cause of this failure may be either that the spraying was not effective in reaching the olfactory mucosa, that the virus of poliomyelitis does not always enter by the nasal mucosa, or that in setting up an inflammatory reaction in the nasal mucosa chemical sprays may destroy virucidal substances normally present in this area. Schultz and Gebhardt (1938 [2]) believe that spraying the olfactory mucosa cannot be effective, unless complete anosmia is produced. In some adults, however, after spraying with 1 per cent. zinc sulphate solution, the sense of smell has not returned after an interval of six months, while poliomyelitis has occurred even after the development of complete anosmia.

References

ALEXANDER, G. L., 1938, Unpublished observations.

ARMSTRONG, C., 1937, Experience with the picric acid spray in the prevention of poliomyelitis in Alabama, 1936. *Amer. J. publ. Hlth.*, XXVII., 103.

ARMSTRONG, C., and HARRISON, W. T., 1935, Prevention of intranasally inoculated poliomyelitis of monkeys by the instillation of alum into the nostrils. *Publ. Hlth. Rep., Wash.*, L., 725.

BÄR, F., 1938, Chemotherapeutische Versuche bei der experimentellen Infektion der Maus mit Lymphogranuloma inguinale. *Klin. Wschr.*, XVII., 588.

CH'I, H. C., 1936, Clinical experiences with local quinine therapy in trachoma. *Chin. med. J.*, L., 1401.

CLIMENKO, D. R., CROSSLEY, M. L., and NORTHEY, E. H., 1938, The protective action of certain sulfanilamide derivatives in experimental influenza infections. *J. Amer. med. Ass.*, CX., 2099.

CONTAT, C., 1938, Chimiothérapie de la poliomyelite aiguë. *Schweiz. med. Wschr.*, LXVIII., 669.

CROSSLEY, M. L., 1938, Drugs of the sulfanilamide family. *Science*, LXXXVII., Supplement, p. 8.

DAVIAUD, and FOURNEAUX, S., 1936, Sur les applications de la valeur anti-infectieuse d'un nouveau dérivé sulfamidé dans le traitement de la maladie de Carré. *Rec. Méd. vét.*, CXII., 653.

Dickerson, V. C., and Whitney, L. F., 1938, Sulfanilamide and prontosil in the treatment of canine distemper. *Proc. Soc. exp. Biol., N.Y.*, XXXVIII., 263.

Dochez, A. R., and Slanetz, C. A., 1938, The treatment of canine distemper with a chemotherapeutic agent, sodium sulfanilyl sulfanilate. *Science*, LXXXVII., 142.

Elyan, M., 1938, Treatment of the common cold. *Brit. med. J.*, *i.*, 146.

Findlay, G. M., and MacCallum, F. O., 1938, Chemotherapy of virus diseases. *Ibid.*, *i.*, 875.

Findlay, G. M., Mackenzie, R. D., and MacCallum, F. O., 1938, A morphological study on the virus of lymphogranuloma inguinale. *Trans. R. Soc. trop. Med. Hyg.*, XXXII., 183.

Gjurić, J., 1938, Neue Wege in der Behandlung des Lymphogranuloma inguinale. *Münch. med. Wschr.*, LXXXV., 335.

Kelson, S. R., 1937, Use of sulphanilamide (para-aminobenzenesulphonamide) in experimental poliomyelitis. *Proc. Soc. exp. Biol., N.Y.*, XXXVI., 718.

Kirk, R., McKelvie, A. R., and Hussein Ahmed Hussein, 1938, Prontosil in the treatment of trachoma. *Lancet*, *ii.*, 994.

Kubitzki, 1938, Beitrag zur Behandlung lokaler und allgemeiner Infektionen und des Lymphogranuloma inguinale mit prontosil. *Dtsch. militärärztlz. Z.*, III., 116.

Kulesza, S., 1937, (Preliminary report on prontosil in the therapy of typhoid). *Medycyna*, XVI., 570.

Levaditi, C., 1938^{1}, L'activité thérapeutique des dérivés benzéniques sulfurés dans les maladies provoquées par les ultravirus. *C.R. Soc. Biol., Paris*, CXXVII., 958.

Levaditi, C., 1938^{2}, Chimiothérapie de la lymphogranulomatose inguinale expérimentale. *Ibid.*, CXXVIII., 138.

Levaditi, C., and Vaisman, A., 1935, Action curative et préventive du chlorhydrate de 4′-sulfamido-2 : 4-diaminoazobenzène et de quelques dérivés similaires dans la streptococcie expérimentale. *Pr. med.*, XLIII., 2097.

Lian, B., 1938, Prontosiltherapie bij trachoom. *Geneesk. Tijdschr. Nederl-Indie.*, LXXVIII., 1058.

Loe, F., 1938, Sulfanilamide treatment of trachoma : preliminary report. *J. Amer. med. Ass.*, CXI., 1371.

MacCallum, F. O., and Findlay, G. M., 1938, Chemotherapeutic experiments on the virus of lymphogranuloma inguinale in the mouse. *Lancet*, *ii.*, 136.

MacIntyre, A. B., and Montgomerie, R. F., 1938, Chemotherapy of virus diseases. *Brit. med. J.*, *i.*, 875.

McKinley, E. B., Acree, E. G., and Meck, J. S., 1937, Sulfanilamide and virus diseases. *Science*, LXXXVII., 43.

Marcus, P. M., and Necheles, H., 1938, Treatment of spontaneous canine distemper with sulfanilamide. *Proc. Soc. exp. Biol., N.Y.*, XXXVIII., 385.

Mauer, G., 1938, Die Wirkung des Trypaflavins bei psittakoseinfizierten Mäusen. *Zbl. Bakt.* Abt. I. Orig., CXLII., 279.

Montel, L. R., and Nguyen-Van-Tho, 1938, Traitement de la maladie de Nicolas-Farre par les dérivés sulfamidés (Rubiazol-Septazine). *Bull. Soc. Franç. Derm. Syph.*, XLV., 652.

Oakley, C. L., 1938, Chemotherapy of virus diseases. *Brit. med. J.*, *i.*, 895..

Rhodes, A. J., and van Rooyen, C. E., 1938, Chemotherapy of virus diseases. *Ibid.*, *i.*, 924.

Ronse, M., 1938, Essai de chimiothérapie dans la chorio-meningite de la souris. *C.R. Soc. Biol., Paris*, CXXVII., 845.

ROSENTHAL, S. M., WOOLEY, J. G., and BAUER, H., 1937, Studies in chemotherapy. VI. The chemotherapy of choriomeningitis virus infection in mice with sulphonamide compounds. *Publ. Hlth. Rep., Wash.*, LII., 1211.

SABIN, A. B., OLITSKY, P. K., and COX, H. R., 1936, Protective action of certain chemicals against infection of monkeys with nasally instilled poliomyelitis virus. *J. exp. Med.*, LXIII., 877.

SCHMIDT, H., and PETER, F. M., 1938, " Advances in the Therapeutics of Antimony." Leipzig.

SCHULTZ, E. W., and GEBHARDT, L. P., 1936, Prevention of intranasally inoculated poliomyelitis in monkeys by previous intranasal irrigation of chemical agents. *Proc. Soc. exp. Biol., N.Y.*, XXXIV., 133.

SCHULTZ, E. W., and GEBHARDT, L. P., 1938 [1], Zinc sulfate prophylaxis. *J. Amer. med. Ass.*, CVIII., 2182.

SCHULTZ, E. W., and GEBHARDT, L. P., 1938 [2], The use of zinc sulfate solution for the prevention of poliomyelitis in man. *Ibid.*, CX., 2024.

SELINGER, E., 1935, Local quinine therapy in trachoma : preliminary report. *Amer. J. Ophthal.*, XVIII., 631.

SHROPSHEAR, G., 1938, Sulfanilamide in treatment of strictures of rectum caused by lymphogranuloma venereum : preliminary report. *Illinois med. J.*, LXXIV., 97.

THOMPSON, A. R., and GREENFIELD, C. R. M., 1938, Chemotherapy in measles and whooping cough : prophylaxis and treatment of complications. *Lancet, ii.*, 991.

TISDALL, F. F., BROWN, A., DEFRIES, R. D., ROSS, M. A., and SELLARS, A. H., 1937, Zinc sulphate nasal spray in the prophylaxis of poliomyelitis. Observation of a group of 4,713 children, age 3–10 years, during an epidemic in Toronto, Canada. *Canad. publ. Hlth. J.*, XXVIII., 523.

WHICHER, A., and SMITH, H. L., 1937, Some observations on the use of prontosil in canine practice. *Vet. Rec.*, XLIX., 452.

AUTHOR INDEX

Acree, E. G., 486, 494
Acres, I. S., 161, 162
Acton, H. W., 76, 85, 86, 98, 99
Adair, F. L., 331, 335
Adams, A. R. D., 193, 199, 212, 218
Adams, R., 304, 311, 313
Adler, S., 298, 302
Advier, 302
Afanador, A., 316, 318
Africa, C. M., 85, 86
Agerty, H. A., 360
Ahringsmann, H., 413, 414
Ainley, A. D., 111, 112, 116
Akashi, K., 67, 74
Akers, N. C., 302
Albert, A., 465, 466, 467, 468
Albright, F., 424, 425
Alcay, 316, 319
Alegret, E., 294, 297
Alexa, E., 126
Alexa, I., 126
Alexander, G. L., 487, 493
Alexandrescu, D., 122, 123
Alfred, E. S. R., 320, 321
Allan, W. B., 388, 389
Allen, J. G., 330, 336, 341, 440
Alliez, J., 292, 296
Allott, E. N., 332, 335, 361, 362
Alms, 10
Altmann, R., 116, 135, 136
Alstead, G., 448, 449
Amberson, J. B., 293, 295
Ambialet, R., 122, 123
Amies, C. R., 151
Amy, A. C., 129, 131
Anderson, H. H., 21, 22, 61, 62, 63, 64, 67, 68, 72, 73, 74, 78, 80, 81, 82, 83, 84, 85, 304, 305, 307, 310, 311, 312, 315, 318
Anderson, J. v. D., 307, 310, 313
Anderson, N. P., 258
Anderson, R. C., 332, 335
Anderson, T., 350, 351, 360
Anderson, T. E., 213, 217
André, E., 302
Angerer, H., 351, 356
Anghelescu, V., 350, 357
Appel, B., 258
Appelbaum, E., 355, 359, 391
Arafa, M. A., 85, 86
Archer, H. E., 446, 447
Archibald, R. G., 54
Archinard, J. J., 433, 435
Arias Aranda, C., 103, 104
Armand-Delille, P. F., 394
Armstrong, C., 492, 493
Arnaud, R., 206, 207, 208
Arnold, E., 268
Arnold, H., 310, 313
Artagaveytia-Allende, R., 183, 186, 188, 198
Ascoli, M., 122, 123
Ashford, B. K., 16, 22, 25, 26, 120, 123
Ashley, J. N., 176, 178
Astrachan, G. D., 266, 268
Attwood, A. M. P., 99, 306, 308, 309, 312
Ayres, S. Jr., 258
Azim, M. A., 13, 15, 22
Azmy, S., 74

Baer, W. S., 484, 485
Baermann, G., 206, 208
Bainbridge, H. W., 99
Bais, W. J., 62, 63
Baize, P., 93, 99
Bakhsh, I., 75, 76
Balcerski, M., 435
Baldridge, C. W., 252, 258
Baldwin, E. R., 285, 293
Ballenger, E. G., 401, 407
Ballif, L., 122, 123, 146
Balteanu, I., 126
Baltzly, R., 23
Balzar, F., 279, 282
Balzer, H., 289, 295
Bambas, L. L., 332, 336
Banerjea, J. C., 292, 293, 295
Banerjea, R., 92, 99, 101, 104
Banerjee, N. G., 150, 151
Bangindo, Z. G. S., 145, 148
Banks, H. S., 394, 395, 396
Bannick, E. G., 424, 425
Bär, F., 491, 493
Baranger, P., 309, 312
Barber, M. A., 150, 151
Barber, H. W., 432
Barbosa, A., 143, 146
Barbour, G. M., 407
Barer, M., 419, 420
Barger, G., 132, 135
Barke, A., 334, 335
Barker, R. H., 332, 335
Barlovatz, A., 157, 158, 192, 196
Barlow, O. W., 337, 341, 368
Barnett, C. W., 266, 268
Barnett, H. J., 334, 335, 444
Barr, A., 416, 418
Barr, J. B., 347, 357
Barron, E. S. G., 456, 460
Barrowcliff, M., 301, 302
Barthelme, F. L., 481
Barton, J. C., 124
Basaca, M., 13, 24
Basmas, J., 338, 389, 424, 425, 444
Basnuevo, J. G., 51, 55
Bass, R., 23
Bastianelli, G., 128, 130, 144, 146
Basu, B. C., 151
Basu, R., 93, 99
Batchelor, R. C. L., 422, 423
Baudet, E. A. R. F., 35, 40
Baudouin, A., 441
Bauer, H., 361, 362, 372, 373, 377, 380, 382, 383, 385, 389, 392, 397, 463, 486, 487
Baumler, C., 2, 5
Becker, S. W., 271
Becker, W., 350, 357
Beerman, H., 256, 259, 269, 270, 283, 284
Bender, W. L., 78, 83
Beneazzo, G., 257, 259
Bennett, S. C. J., 177, 178
Benoit, G., 116, 135, 136
Bentley, C. A., 2, 5
Bequignon, R., 459, 462
Berck, M., 418, 419
Beretervide, J. J., 66, 67
Berg, H., 206, 208
Berg, S., 438, 440
Berger, W., 413, 414
Bergman, O., 8, 9
Bernard, L., 293, 295
Bernard, R., 255, 259
Bernstein, S. S., 354, 357, 394, 395, 396
Berny, P., 134, 135. 317, 318
Bertaccini, G., 317, 318
Bertheim, A., 79, 84, 211, 214, 217
Berthier, J., 292, 295
Bertrand, C., 46, 48, 55
Béthoux, L., 413, 414
Biekand, G. R., 252, 259
Bieling, R., 470, 473
Biggam, A. G., 15, 22, 85, 86
Bigler, J. A., 436, 437
Bigot, A., 316, 318
Binz, A., 274, 469
Bird, W., 110, 117
Birkeland, J. M., 459, 461
Birkhaag, K. E., 480, 481, 482
Birnbaum, G., 247, 259
Bishop, A., 68, 69
Bispham, W. N., 122, 123, 145
Blackie, W. K., 129, 130
Blake, F. G., 354, 358, 429, 430

Blake, K. W., 413, 414, 432, 435, 436, 443
Blakely, E., 254, 260
Blatt, M. L., 408
Bliss, E. A., 335, 336, 341, 345, 352, 354, 357, 358, 359, 381, 390, 396, 412, 421, 424, 426, 427, 428, 441, 442, 443, 453, 455, 459, 460, 462
Bloch, E., 410, 412
Bloch-Michel, H., 350, 357
Bloom, W., 480, 481
Blumgart, H. L., 413, 414
Boak, R. A., 398, 409
Bode, H. G., 350, 357
Bodin, E., 250, 251, 259
Boerio, V., 126
Bogert, N., 465, 468
Bohlman, H. R., 420, 421
Böhm, A., 286
Bohnstedt, R. M., 215, 217
Borst, J. G. G., 438, 440
Bossé, G., 427
Bosse, O. A., 346, 357
Bourgeois, P., 292, 295
Bovet, D., 114, 116, 134, 135, 136, 188, 196, 323, 329, 378, 381, 382, 391, 403, 409, 410, 412, 449, 454, 463
Bowie, F. J. T., 405, 407
Boyd, J. S. K., 129, 130
Boyd, T. C., 98, 99
Boyer, H., 403, 407
Boze, I. B., 75, 77
Bozicevich, J., 15, 24, 42
Bozzolo, 2, 6
Braasch, W. F., 474, 478
Brahmachari, P., 94, 99, 153, 154
Brahmachari, U. N., 90, 92, 94, 99, 101, 104
Brainin, W., 402, 407
Brandaleone, H., 484, 485
Branham, S. E., 368, 382, 383, 384, 390, 392, 393, 394, 396, 397, 407, 463
Breen, J., 350, 357
Breinl, A., 192, 196
Bremer, O., 468, 469
Brennerman, J., 353, 354, 357
Briercliffe, R., 142, 143, 146
Brimint, E., 192, 198
Brindley, W. H., 60, 63
Britton, C. J. C., 390, 414, 419, 420, 421, 436, 437
Bromfield, R. J., 153, 154
Broom, H. W., 19, 22
Broom, J. C., 231
Broughton, L. B., 8, 9
Broutman, J., 292, 295
Brown, A., 479, 495
Brown, A. E., 425
Brown, D. B., 347, 357, 411, 412, 439, 440
Brown, H., 224, 226, 281, 283, 469
Brown, H. C., 76, 77, 231, 253, 259
Brown, H. W., 5, 6, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 29, 30, 34, 36, 41
Brown, J. L., 356, 357
Brown, J. W., 390
Brown, J. Y., 130
Brown, M. T., 393, 396
Brown, P. W., 78, 79, 84
Browning, C. H., 175, 176, 178, 181, 182, 188, 190, 191, 196, 197, 202, 207, 208, 229, 231, 284, 285
Browning, P., 183, 186, 188, 197
Brownlee, I. E., 384, 391, 436, 437, 455, 457, 458, 463
Brulé, M., 402, 407
Brunschwich, M., 361, 362
Brunsting, L. A., 433, 434
Bryant, J. B., 361, 362
Buchsbaum, R., 480, 481
Buchtel, H. A., 399, 407, 417, 418, 475, 478
Bucy, P. C., 431, 435, 436
Bunker, H. A., 221
Burchell, H. B., 471, 473
Bürger, L., 431
Bürgers, 454, 460
Burgess, N., 288, 295
Burn, J. H., 244
Burthiault, R., 348, 359
Butler, A. M., 344
Butler, C. L., 470, 473
Buttle, G. A. H., 111, 112, 113, 114, 115, 116, 295, 323, 328, 332, 336, 337, 341, 345, 357, 361, 362, 364, 366, 367, 368, 369, 371, 372, 373, 375, 376, 377, 380, 381, 383, 384, 385, 387, 390, 392, 396, 409, 410, 412, 415, 418, 452, 453, 454, 460, 482, 483
Butz, L. W., 8, 9
Byrne, P. S., 388, 390

CAFFEY, J., 282
Caius, J. F., 10, 12, 25, 26, 27, 30
Calandruccio, F., 3, 6
Caldwell, E. L., 13, 14, 17, 23
Caldwell, J. R., 388, 390
Cameron, G. R., 29, 30
Cameron, W. M., 88, 101
Campbell, A. D., 252, 259
Campbell, C. M., Jr., 436, 437
Canalis, A., 128, 130, 144, 146
Cannon, A. B., 239, 243, 256, 259, 281, 282
Cannon, P. R., 429, 430
Cardoso, H., 309, 312
Carey, A. M., 344, 350, 351, 352
Carey, B. W., 355, 357, 394, 396, 401, 407
Carman, J. A., 422
Caronia, G., 87, 88, 93, 99
Carpenter, C. M., 398, 407, 409
Carpenter, C. P., 370, 382
Carr, J. L., 437
Carr-Fraser, W. A., 4, 6
Carroll, G., 475, 477, 478
Castellani, A., 85, 96
Castelli, 215, 217
Castle, W. B., 26, 27
Catanei, A., 122, 123
Cathcart, E. P. P., 249, 262
Caventou, 107, 117
Cawston, F. G., 46, 48, 51, 54
Cerf, M., 255, 259
Cerqueira, P., 304, 307, 310, 312
Chabrun, J., 93, 99
Chambers, E. R., 268
Chano, D., 125, 126
Chao, S. S., 193, 198, 223, 227
Chapman, C. W., 242, 243, 244
Charron, E., 119
Chatterjee, B., 484, 485
Chatterjee, R. N., 90, 99, 103, 104
Chatterji, S. P., 315, 319, 320, 321
Chaudhuri, R. N., 95, 100, 153, 154
Chaudhury, S. G., 96, 99
Chelarescu, M., 122, 123, 146
Chen, K. K., 332, 335, 337, 341
Chen, M. Y., 63, 78, 80, 84
Chesley, L. C., 445, 447
Chesney, A. M., 470, 473
Chesterman, C. C., 158
Cheu, S. H., 56, 57
Chevallier, P., 255, 259
Ch'i, H. C., 492, 493
Child, R., 70, 74
Chin, K., 139, 146
Chin, T. L., 483
Chinn, B. D., 414
Chorine, V., 317, 319
Chopra, G. S., 76, 77
Chopra, R. N., 8, 9, 15, 23, 32, 34, 59, 60, 62, 63, 66, 67, 76, 77, 81, 82, 84, 90, 91, 96, 97, 98, 99, 102, 104, 120. 139, 140, 141, 146, 151, 152, 153, 154
Chowdhury, J. C., 76, 77
Chowhan, J. S., 104
Christensen, B. V., 15, 22, 31, 34
Christison, M. H., 167, 274, 275
Christophers, S. R., 137, 138, 146, 183, 186, 225, 226
Christopherson, J. B., 43, 45, 54
Chu, C. F., 93, 99
Chu, H. J., 484, 485
Chu, H. L., 49, 55
Chung, H. L., 49, 55
Citron, H., 192, 197
Ciuca, M., 122, 123, 126, 145, 146
Clapham, P. A., 16, 22
Clark, A., L. 474, 478
Clark, A. R., 455, 459, 461
Clarke, A. J., 225, 226
Clarke, H. C., 150
Clason, F. P., 360
Clemence, L. W., 382, 469
Clement-Simon, 251, 259
Clemo, G. R., 10. 12

Clifton, W. M., 437
Climenko, D. R., 488, 493
Cluzet, 318, 319
Cochrane, R. G., 320, 321
Coggeshall, L. T., 152
Coghlan, B. A., 172, 173
Cohen, A., 111, 113, 116, 163, 164, 165, 166, 167, 214, 217, 218, 221, 224, 226
Cohen, J. B., 175, 176, 178, 188, 197, 202, 468
Cohen, S. S., 470, 473
Cohn, A., 399, 407
Cokkinis, A. J., 400, 401, 406, 407, 416, 417, 418, 423, 424, 425, 445, 447
Cole, H. I., 301, 302, 306, 307, 309, 312
Cole, H. N., 240, 244, 250, 259
Colebrook, L., 331, 335, 345, 346, 347, 349, 352, 357, 410, 412, 432, 445, 446, 448, 453, 454, 455, 461
Collier, W. A., 166, 167, 289, 295, 464
Colston, J., 399, 407
Coman, D. R., 460, 461
Connor, W. H., 271
Contat, C., 492, 493
Conte, M., 357
Constantinesco, N., 126, 212, 217
Cook, E. N., 399, 407, 417, 418, 474, 478
Cook, S. S., 246, 259
Cooper, F. B., 346, 358, 364, 372, 380, 383, 384, 385, 390, 391, 423, 425, 458, 459, 461
Cooper, G. A., 264, 268
Cooper, K. E., 175, 176, 178
Copeman, W. S. C., 290, 295
Cormia, E. B., 248, 259
Cornwall, L. H., 221
Corson, J. F., 198, 205, 208
Cossio, R., 178, 179
Coulthard, E. C., 18, 22, 69, 70
Cousin, E., 220, 222
Cownley, A. J., 60, 64
Cox, H. R., 492, 494
Craddock, A. L., 395, 396
Cram, E. B., 35, 41
Craven, E. B., 258, 259
Crawford, T., 394, 395, 396
Crean, T. F., 394, 396, 400, 401, 407
Cretcher, L. H., 473
Crivetz, D., 357
Crosley, S. S., 281, 283
Crossley, M. L., 488, 493
Cruickshank, J. C., 423, 425
Cruz, A. O., 311, 312
Cruz, M. C., 308, 312
Cuilleret, P., 286
Curtillet, A., 61, 64
Cuthbert, J. C., 349, 357
Cutting, W. C., 330, 332, 333, 334, 336, 337, 338, 340, 341, 342, 436, 437, 444
Czarnetzky, E. J., 481, 482

DALE, H. H., 61, 62, 63, 243, 244
Dalimier, R., 63
Dalldorf, G., 201, 202
Dangerfield, W. G., 171, 173, 203, 208, 221
Dantec, 220, 222
Das Gupta, B. M., 66, 67, 103, 104, 146, 152
Das Gupta, J. M., 92, 99, 103
Da Silver, R. A. D., 301, 302
Daubney, R., 35, 40
Daude, J., 159, 160
Daver, M. B., 484, 485
Davesne, J., 361, 362
Davey, T. H., 28, 30
Daviaud, 489, 493
David, N. A., 17, 21, 22, 61, 64, 72, 73, 74, 81, 83, 84, 85
Davies, R. M., 78, 84
Dawson, W. T., 110, 116, 473
De, N. K., 308, 312
De, P., 62, 63
De, R. K., 144, 146
Dean, A. L., 301, 302
De Blieck, L., 35, 40
De Buck, A., 128, 131
Deeks, W. E., 85, 86
Dees, J. E., 399, 407
Defries, R. D., 495
Degotte, J., 316, 318
De Langen, C. D., 129, 131, 138, 139, 146
Delanoë, E., 316, 318
Demanche, L., 134, 136
De Marqueissac, H., 172, 173
Demidova, L. W., 115, 116, 117
Depierre, F., 384, 391, 454, 463
Desoille, 257
Despois, R., 323, 329, 381
Devine, J., 185, 186
Devolf, H., 258
De Vries, S. I., 438, 440
Dewing, G. T., 371, 380, 390, 412, 483
Díaz de Leon, A., 152
Dickerson, V. C., 489, 493
Di Cristina, G., 86, 99
Dienes, L., 424, 425
Dikshit, A. B., 303, 307
Dikshit, B. B., 103, 104, 129, 130, 131, 303, 307, 310, 312
Dimitry, T. J., 308, 312
Dimson, S. B., 395, 396
Dirscherl, W., 112, 116
Discombe, G., 446, 447, 448
Dixon, H. B. F., 128, 131
Dixon, R. S., 268
Dobell, C., 62, 63, 68, 70, 197
Doble, J., 344
Dochez, A. R., 370, 380, 489, 493
Dolan, L. P., 478
Domagk, G., 322, 328, 339, 341, 349, 357, 363, 367, 368, 380, 381, 383, 384, 390, 407, 409, 410, 412, 421, 437, 452, 454, 459, 461, 483
Domes, A., 405, 409
Dosquet, H., 289, 295
Dostrowsky, A., 102, 104
Douglas, R. G., 430, 481, 482
Douglass, M., 201, 202
Douthwaite, A. H., 356, 357
Dowling, P. C., 268
Dozzi, D. L., 448, 449
Dressel, O., 316, 322, 329
Dubois, A., 168, 170, 190, 197, 318, 320, 321
Duke, H. L., 191, 197, 206, 208, 234, 235
Dundas, J., 424, 425
Dunlop, D. M., 479
Dunlop, J. L., 253, 259
Dunn, C. G., 483
Durel, P., 257, 259, 357, 400, 402, 408, 441
Durham, F. M., 241, 244
Dwan, P. F., 354, 360
Dyckeroff, H., 72, 74
Dyer, F. J., 468
Dyer, H. A., 211, 214, 218, 223, 227, 268, 277, 278
Dyke, S. C., 389, 390, 391
Dyke, W. J. C., 238, 244, 337 341

EAGLE, H., 278
Eaton, J. C., 446, 448
Eck, M., 356, 360
Ecker, E. E., 480, 482
Eckler, C. R., 271
Edwards, J. T., 206, 208
Egglestone, C., 61, 63
Ehrlich, P., 78, 79, 84, 126, 131, 193, 197, 210, 211, 217, 226, 238, 264, 268
Eichholtz, F., 129, 131
Eisenberg, P., 324, 328
Ejercito, A., 125, 126
El Betash, M. L., 47, 55
El Biblawi, A., 74, 75
Eldahl, A., 395, 396
El Din, M. S., 47, 55
Elder, O. F., 401, 407
Elias, S., 103, 104
Ellingworth, S., 175, 176, 178, 188, 197
Elyan, M., 487, 493
Emerson, E. K., 330, 332, 333, 334, 336, 337, 338, 340, 341, 342, 344, 436, 437, 444
Emerson, G. A., 303, 304, 305, 307
Emmett, J. L., 481, 482
Engel Bey, 309, 312
Ensbruner, G., 256, 261
Epstein, D., 60, 51, 63
Epstein, E., 82, 84, 247
Epstein, N. N., 252, 254, 259, 260, 267, 268
Erhardt, A., 52, 55
Erskine, D., 389, 391, 405
Euler, H. E., 434
Evans, G. M., 388, 390
Evans, J, T. R., 168, 169, 177, 178

FAGERSTROM, D. P., 424, 426
Fairbairn, H., 157, 159, 171, 172, 173

Fairley, N. H., 153, 154
Fakhry, A., 45, 54
Falconer, E. H., 267, 268
Fargher, R. G., 46, 54, 90, 99
Farrell, J. I., 398, 408
Faust, E. C., 14, 22, 86
Feinstone, W. H., 331, 335, 339, 341, 368, 372, 378, 381, 385, 390, 392, 393, 396, 410, 412
Feldt, A., 197, 288, 295
Felke, H., 399, 400, 404, 408
Fenton, W. J., 412
Fermoselle-Bacardi, J., 51, 55
Fernández, G., 304, 307
Fernández, J. M. M., 314, 318
Fernández, L. J., 402, 408
Fernández, R. F., 402, 408
Fernndo, P. B., 143, 147
Fernan-Núnez, M., 152, 154
Field, F. E., 27, 30
Field, J. W., 125, 126
Field, 140
Filliol, L., 259
Findlay, G. M., 28, 29, 30, 201, 202, 253, 259, 487, 491, 493, 494
Finkelstein, R., 459, 461
Finklestone-Sayliss, H., 436, 437, 454, 459, 461
Finland, M., 388, 390
Finney, J. O., 433, 435
First, A., 272
Fischer, C., 405, 408
Fischer, O., 145, 147
Fischer, W., 205, 208, 234, 235
Fischl, L., 200, 202
Fischl, V., 116, 146, 147, 167, 181, 194, 195, 197, 198, 200, 202, 213, 218, 222, 223, 226, 227, 290, 296
Fisher, B. H., 315, 318
Fitch, W. K., 326, 329
Fitzgerald, G. H., 67
Fitz-Hugh, T., Jr., 440
Flake, C. G., 355, 357
Flandin, C., 79, 84, 309, 312
Fleming, A., 389, 391
Fleming, G. B., 394, 395, 396
Fleming, J. B., 356, 357
Fletcher, W., 125, 126
Fleury, O., 219, 222
Fockler, 252, 261
Forester, H. R., 266, 268
Foerster, O. H., 266, 268
Foerster, 322, 329
Follis, R. M., 295, 296
Forbes, J. C., 29, 30
Fordos, M, J., 288, 295
Forst, A. W., 279, 282
Foster, F. P., 425
Foster, G. E., 371, 380, 390, 412
Foster, W. D., 35, 41
Foulis, M. A., 347, 357
Fourche, J. A., 204, 207, 208
Fourneau, E., 78, 84, 132, 133, 159, 160, 163, 170, 173, 366, 371, 372, 373, 375, 377, 378, 381, 385, 391, 424, 425
Fourneaux, S., 489, 493
Fournier, A., 102, 105
Fournier, L., 286
Fowler, R. H., 360
Fox, C. L., Jr., 445, 448
Foy, H., 140, 143, 147
Francis, A. E., 413, 414, 468
Francke, M., 126
Franke, 187, 197
Frauenberger, G. S., 360
Frazier, C. N., 304, 307
Frei, W., 249, 259
Freusberg, O., 431
Fréville, L. H. F., 316, 317, 318
Friedberger, E., 214, 217
Frimodt-Möller, C., 293, 295
Fromm, E., 371, 372, 381
Frost, L. D. B., 447, 448
Fry, R, M., 294, 295, 348, 358
Fulghum, J. E., 353, 358
Fuller, A. T., 323, 329, 331, 333, 335, 342, 343, 344, 461, 474, 478
Fulton, J. D., 115, 116, 147, 225, 226

GABALDON, A., 80, 82, 84
Gaddum, J. H., 241, 244
Gailliot, P., 323, 329, 381
Gaisford, W. F., 388, 390
Gallagher, J. R., 353, 358
Galliot, A., 257, 259
Galloway, I., 204, 209
Ganguli, P., 138, 147
Ganguli, S. K., 139, 146
Gantenberg, R., 352, 353, 358
Garbade, F. A., 110, 116
Garcia, F., 11, 12
Garin, C., 33, 34
Garland, H. G., 291, 295
Garrison, H. F., 33, 34
Garrod, L. P., 330, 335, 346, 349, 455, 458, 461
Garvin, C. F., 441
Gaskill, R. C., 124
Gaté, J., 286
Gaunt, R., 468
Gaunt, W. E., 171, 173, 204, 208, 221
Gauthier-Villars, P., 290, 297
Gay, F. P., 455, 459, 461
Gebhardt, L. P., 492, 493, 494, 495
Gehrt, J., 393, 396
Geiger, J. C., 344
Geiling, E. M. K., 429, 430
Gélis, A., 288, 295
Gelman, S., 397, 426, 428
Gelmo, P., 323, 329
Gentzkow, C. J., 141, 147
Ghalioungi, P., 15, 22
Ghosh, A. B., 75, 76, 77
Ghosh, B. N., 62, 63, 141, 148
Ghosh, S., 75, 77, 90, 99
Gibberd, M., 352, 358
Gibbs, E. M., 108, 116, 117
Giemsa, G., 109, 111, 116
Gilbert, A. P. W., 145
Gilbrin, E., 290, 297
Gillies Annan, W., 401, 408
Giordano, M., 74, 75
Giovannola, A., 48, 54
Girard, A., 325, 329, 373, 380, 381, 391, 393, 406, 408, 456, 461, 483,
Gjuné, J., 490, 494
Glaser, L., 122, 123, 146
Gley, P., 325, 378, 381, 446, 448, 456, 461, 482, 483
Glowazky, F., 225, 227
Gluschki, A., 11, 12
Glyn-Hughes, F., 162, 186, 273, 274
Gmelin, L., 351, 358
Goissidet, P., 323, 329, 363, 381
Goldenburg, M., 352, 358
Goldstein, O., 253, 259
Gompertz, J. J., 438, 440
Gonder, R., 194, 197, 215, 217
Gonthier, B., 33, 34
Goodman, M. H., 433, 435
Goodson, J. A., 110, 112, 113, 117
Goodwin, M. H., Jr., 152
Gornall, F. H., 301, 302
Gougerot, H., 282
Gough, G. A. C., 166, 167
Goulding, E., 302
Gourdon, E., 413, 414
Graffar, M., 251, 253, 255, 260
Gralka, R., 478
Gram, H. C., 476, 478
Grassis, G. B., 3, 6
Grau, C. A., 66, 67
Gray, W. H., 46, 54, 90, 91, 99, 132, 136, 323, 337, 341, 345, 364, 366, 367, 369, 375, 380, 381, 383, 390, 396, 412, 452, 461, 483
Green, R., 109, 117, 141, 147 149, 151
Green, T. H., 252, 261
Greenbaum, S. S., 279, 282, 284
Greenberg, D., 395, 397
Greene, M. R., 287, 297
Greenfield, C. R. M., 492, 495
Greenthal, R. M., 442
Grigorowsky, A. M., 114, 117, 137, 147
Grillo, J., 232, 233
Grimes, C., 318, 319
Groen, J., 438, 440
Gross, P., 346, 358, 364, 372, 380, 383, 384, 385, 390, 391, 423, 425, 458, 459, 461, 462
Groues, P., 413, 414
Groulade, 361, 362
Gruhzit, O. M., 32, 34, 264, 265, 266, 268
Grütz, O., 323, 329, 404, 408
Gubler, 249, 260
Gueffroy, W., 332, 335
Guénot, L., 286
Guha, P. R., 97, 99
Guirdham, A., 354, 358
Gulati, R. I., 57
Gulbransen, R., 175, 176, 178, 188, 194, 197, 200, 202, 207, 208, 229, 230, 231, 284, 285, 468

Gunderson, M. F., 361, 362
Gupta, A. K. D., 91, 99
Gupta, J. C., 60, 63, 76, 77, 91, 99
Guttman, P., 126, 131
Guyomar'ch, 157, 158

Habs, H., 361, 362
Hac, L. R., 335
Hadley, P., 387, 391
Hageman, P. O., 340, 341, 353, 358, 429, 430, 432, 435, 436, 443
Haines, R., 75, 77
Halawani, A., 66, 67
Haldar, K. C., 101, 104
Hall, M. C., 1, 3, 13, 22, 27, 30, 31, 34, 35, 41
Halpern, B. N., 338, 341, 363, 381
Hamon, V., 378, 382
Hannah, R. H., 395, 396
Hanns, A., 448, 449
Hanschell, H. M., 401, 405, 406, 408, 422, 423
Hanson, K. B., 33, 34
Hanzlik, P. J., 279, 280, 282, 283
Harbhagwan, 153, 154
Harrie, P, A., 402, 408
Harris, J. S., 334, 335
Harrison, L. W., 251, 260, 405, 408
Harrison, W. T., 243, 244, 246, 262, 492, 493
Hartfall, S. J., 291, 295
Hartl, K., 351, 358
Hartmann, A. F., 334, 335, 444
Harvey, A. M., 442, 443
Harwood, P. D., 21, 22, 23
Haselden, C. S., 361, 362
Hassan, A., 28, 30, 49, 54, 55
Hasskó, A., 195, 197, 200, 202, 203, 216, 217, 232
Hata, S., 78, 84, 264, 268
Hatcher, R. A., 61, 63
Haugk, 399
Hausheer, W. C., 2, 6
Havard, R. V., 354, 359, 446, 448
Haveaux, G., 204, 207, 208
Hawking, F., 160, 162, 188, 189, 191, 192, 193, 194, 195, 197, 199, 220, 221, 222, 236, 324, 325, 339, 341
Hawley, P. L., 407
Haworth, R. D., 10, 12, 75, 77
Hay, D. C., 142, 147
Hayes, S. N., 348, 358
Hayward, E. W., 103, 104
Heaf, F., 295
Hecht, G., 137, 139, 141, 142, 147, 148
Heidelberger, M., 323, 329, 363, 381
Heinild, S., 291, 296
Heintzelmann, J. H. L., 387, 391
Helmholz, H. F., 333, 335, 398, 408, 417, 418, 474, 476, 477, 478, 479
Helmy, M. M., 28, 30
Hemmings, A. W., 340, 434
Henderson, L. H., 130, 131
Hendrix, H., 118, 119, 168, 170
Hennelly, T. J., 160, 162, 220, 221, 222
Henrard, C., 234, 235
Henry, T. A., 77, 108, 110, 111, 112, 113, 116, 117, 132, 136, 320, 321
Hering, E. R., 401, 408
Herliuson, H., 356, 358
Herrold, R. D., 401, 408
Hesseltine, H. C., 335
Hetherington, D. C., 484, 485
Hewer, C. L., 446, 448
Hicks, E. P., 125, 126, 141, 147
Hill, R. A., 152
Hillemand, P., 402, 407
Hillenbrand, K., 8, 9
Hilz, K., 35, 41
Hinault, V., 292, 296
Hirschfelder, A. D., 201, 203, 468
Hirst, G. K., 360
Hoare, C. A., 231
Hoare, E. D., 455, 461
Hobson, F. G., 395, 396
Hodgkin, 140
Hoerlein, H., 127, 131, 322, 329, 452, 461
Hoffmann, S. J., 402, 408
Hofmann, W., 460, 463
Hogan, B. W., 431
Hogarth, J. C., 354, 358
Hogue, M. J., 80, 84
Holling, H, E., 475, 479
Holtzman, M., 438, 440
Homma, H., 177, 179
Hornby, H. E., 168, 169
Howard, H. H., 25, 27
Howkins, J., 436, 437
Hryntschak, T., 476, 479
Huber, H. B., 415, 418
Huc, H., 350, 358
Hudson, E. H., 281, 283, 291, 296
Hügel, G., 90, 101,
Huguenin, P., 316, 319, 350, 359
Hullstrung, H., 431
Humphreys, R. M., 104
Huntenberg, W., 108, 117
Huntington, R. W., Jr., 458, 461
Hurst, A. F., 254
Hurwitz, E., 310, 312
Hussameddin, 28, 30
Hussein Ahmed Hussein, 494
Hutcheson, A. C., 51, 54
Hutchison, A., 422, 423
Hykes, D. E., 369, 381, 468, 469
Hykes, O. V., 369, 381, 468, 469

Iensch, H., 177, 178
Imhäuser, K., 349, 358
Ireland, F. A., 251, 260
Irgang, S., 253, 260
Isanos, M., 122, 123, 146
Itagaki, S., 56, 57

Jackson, H. W., 391
Jacobs, H. H., 456, 460
Jacobs, J., 248, 260
Jacobs, W. A., 323, 329, 363, 381
Jacoby, A., 423
Jacovidès, 63
Jadassohn, W., 248, 256, 260
Jadin, J., 195, 197
Jahnel, F., 284, 285
James, S. P., 120, 122, 123, 130, 131, 145, 147
James, W. M., 85, 86
Jamot, E., 156, 158
Janeway, C. A., 441, 442
Jankelson, I. R., 258
Janney, J. H., 474, 479
Janssens, P. G., 191, 197
Jarvis, O. D., 139, 147
Jaubert, A., 448, 449
Jauerneck, A., 332, 335
Jausion, H., 403, 408
Jdan-Pushkin, M. N., 306, 307
Jennings, G. H., 394, 396, 439, 440, 442
Jessner, M., 240, 244
Jewsbury, E. C. O., 394, 396
Jirruch, E. A., 429, 430
Joannidès, N. Z., 102, 104
Johnson, A. G., 389, 391
Johnson, S. H., 403, 408
Johnson, T. B., 12, 22
Johnston, J. M., 471, 473
Johnstone, H. G., 17, 22, 56, 57, 73, 74
Johnstone, R. W., 348, 358
Jolly, G. G., 103, 104
Jones, H. W. E. 424, 425
Jones, T. D., 433, 435
Jordan, J. W., 267, 268
Jouatte, D., 302,
Jouve, A., 292, 296
Justin-Besançon, L., 257, 262

Kalló, A., 291, 296
Kallós, P., 248, 260
Kallós-Deffner, L., 248, 260
Kan, H. C., 49, 54
Kappel, L., 477, 478
Karamchandani, P. V., 103, 104
Karelitz, M. B., 249, 260
Karunaratne, W. A. E., 29, 30
Kawai, S., 294, 297
Kehar, N. D., 139, 147
Kehler, 10, 12
Keil, E., 310, 312
Keim, H. L., 249, 260
Kellner, K., 323, 329, 452, 461
Kellogg, F., 260
Kelson, S. R., 486, 494
Kemkes, B., 470, 473
Kemp, H. A., 458, 461
Kemp, J. E., 281, 283
Kendrick, J. F., 32, 34

Kenney, J. R., 471, 473
Kenny, M., 331, 335, 346, 357, 415, 416, 418, 445, 448, 455, 461
Kerr, W. K., 252, 259, 260
Kessel, J., 72, 75
Khalil, M., 45, 47, 48, 49, 50, 54, 55
Khaw, O. K., 50, 55, 56, 57
Kikuth, W., 92, 99, 135, 136, 138, 147, 153, 154, 483
King, H., 111, 112, 113, 116, 163, 164, 165, 166, 167, 183, 186, 214, 217, 218, 221, 224, 238, 244
Kingsbury, A. N., 142, 147
Kingsbury, J., 297
Kinsella, R. A., 345, 359
Kirk, R., 491, 492, 494
Kirk, T. S., 484, 485
Klarer, I., 324, 329, 367
Klee, P., 352, 353, 358
Klein, E., 415, 418
Klein, J. R., 334, 335
Kleine, F. K., 206, 208, 234, 235
Klose, G., 411, 412, 415, 418
Klotz, J. D., 29, 30
Knowles, R., 66, 67
Koch, E. W., 64, 72, 73, 74, 81, 83, 84
Kochman, M., 20, 22
Koetschett, P., 163
Kohn, 441
Kollath, W., 52, 55
Kolmer, J. A., 63, 224, 226, 243, 244, 280, 281, 283, 469, 479, 482
Komp, W. H. W., 150, 151
Kondi, A., 140, 143, 147, 153, 154
Konstam, G., 355, 360
Kornblith, B. A., 423
Kotbra, J., 194, 197
Kothe, H., 322, 329
Kouri, P., 51, 55
Kousa, 102, 104
Kramer, B., 287, 297
Kramer, W., 350, 358
Krasnjansky, M. W., 102, 104
Krassnoff, D., 286, 456, 462
Krauel, G., 440
Krause, F., 431
Kreidler, W. A., 391
Kremens, A., 272
Krishnan, K. V., 98, 100
Kritschewski, I. L., 115, 116, 117, 231, 232, 233
Krouch, M., 119
Krumholz, B., 257, 262
Kubitzki, 490, 494
Kubota, S., 9
Kuhn, P., 47, 56
Kuhs, M. L., 227, 228, 229, 273
Kulchar, G. V., 249, 262, 266, 268
Kulesza, S., 492, 494
Kunert, H., 198
Kung, J. C., 49, 54
Kurotchin, T. J., 483
Kuznetzov, V. N., 306, 307

Labernadie, V., 308, 312
Lacomme, M., 348, 358
Lagrossa, M., 311, 312
Laidlaw, P. P., 68, 70
Lake, G. C., 64
La Lande, W. A., 8, 9
Lamb, C. A., 254, 260
Lambert, S. M., 27, 30, 32, 34, 257, 260
Lamson, P. D., 4, 5, 6, 7, 9, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 31, 34 ,36
Landon, J., 388, 391
Landsteiner, K., 248, 260
Lane, C., 2, 6, 25, 26, 27, 33, 34
Lane, F. W., 12, 22
Lang, K., 171, 173
Lanier, W. D., 424, 425
Lapp, C., 108, 117
Laqueur, B., 102, 104
Lara, C. B., 304, 307, 308, 312
Latto, C., 388, 391
Latvier, A. R., 242, 244
Lauf, R., 248, 260
Lauffer, P. G. I., 468
Launoy, L., 46, 55, 156, 158, 168, 169, 188, 197, 205, 208, 219, 222, 229, 230
Lauppe, F., 268
Laurent, R., 158
Law, R. G., 33, 34
Lawrow, A. R., 102, 104
Lawson, H. A., 26, 27
Layton, W. C., 360
Lazarus, A. S., 480, 482
La Zerescu, V., 357
Leake, C. D., 21, 22, 61, 62, 63, 67, 68, 73, 74, 76, 77, 78, 80, 83, 84, 85, 304, 305, 307, 311
Lebeuf, F., 289, 296
Ledentu, G., 159, 160
Le doux, H. A., 139, 148
Lee, C. D., 48, 49, 55, 93, 99
Lee, H. M., 332, 335
Lees, R., 422, 423
Léger, M., 318, 319
Leggate, J., 315, 319
Lehman, A. J., 280, 282
Leibly, F. J., 62, 64
Leinfelder, P. J., 157, 158
Lemke, R., 431
Lemon, W., 251, 260
Le Moult, 33, 34
Leonard, C. S., 193, 195, 198, 211, 223, 227, 268, 277, 278
Leonard, V., 12, 23
Lepine, P., 316, 317, 319
Lereboullet, P., 93, 99
Lerman, J., 255, 260
Le Roux, P. L., 177, 178
Lescher, F. G., 292, 296
Leskien, I., 361, 362
Lester, H. M. O., 162, 167, 171, 173, 177, 178, 192, 197, 273, 274
Lestocquoy, 395
Leulier, A., 289, 296
Leupold, F., 189, 198
Levaditi, C., 204, 209, 212, 217, 279, 280, 283, 285, 286, 322, 325, 329, 363, 373, 375, 378, 381, 386, 391, 393, 396, 399, 406, 407, 408, 422, 449, 452, 453, 455, 456, 457, 458, 459, 461, 462, 482, 483, 486, 487, 490, 491, 494
Le van Phung, 321
Le van Trien, 316
Levin, E. A., 271
Levinson, M., 449
Levy, C. S., 433, 435
Levy, R., 322, 470, 473
Lewin, E. M., 254, 260
Lewis, B., 477, 478
Lewis, M., 364, 380, 385, 390, 423, 424, 425, 458, 461
Ley, L., 348, 358
Li, T. Y., 49, 55
Lian, B., 491, 494,
Lignos, A., 93, 99
Lin, L. C., 95, 100
Lindsay, J. W., 28, 30, 360
Linnæus, C., 117, 167
Linnell, N. H., 465, 466, 467, 468
Linser, K., 356, 358, 399, 409
Lippett, H., 468, 469
Lisganooa, A. W., 232, 233
Litchfield, G. T., 342, 344
Lloyd, J. H., 413, 414
Lloyd, R. B., 96, 99
Lloyd, V. E., 389, 391, 405, 409
Loe, F., 491, 494
Löhe, H., 404, 405, 409
Lohr, W., 484, 485
Long, P. H., 331, 336, 345, 352, 354, 358, 359, 360, 381, 390, 396, 421, 426, 427, 428, 441, 442, 443, 444, 453, 455, 459, 460, 462
Longcope, W., 350, 351, 354, 359, 442, 443
Loré, A., 157, 158
Lorenz, W. F., 273
Louis, 388, 391
Lourie, E. M., 123, 183, 186, 188, 189, 198, 212, 213, 217
Lovelace, J. W., 355, 354
Low, G. C., 95, 100
Lowe, J., 308, 312
Ludford, R. J., 314, 319
Ludovici, H. L., 142, 147
Lüdtke, H., 7
Lund, N., 294, 296
Lynch, H. J., 15, 22, 31, 34
Lyon, D. M., 475, 479
Lyth, H., 354, 359

MacAlister, C., 484, 485
McAlonan, W. T., 268
McBroom, J., 412
MacCallum, F. O., 201, 202, 487, 491, 492, 493, 494
McConnell, J. S., 29, 30
MacCormac, H., 283, 284
McDonagh, J. E. R., 43
McDonald, H. P., 401, 407

McElligott, G. L. M., 400, 406, 407
Macfie, J. W. S., 110, 112, 113, 117
MacGilchrist, A. C., 110, 117
McGuire, J. P., 439, 440
McGuire, P. R., 439, 440
MacIntosh, R., 354, 359, 394, 396, 422, 424, 425
McIntosh, R. L., 268
MacIntyre, A. B., 4, 361, 489, 494
McKelvie, A. R., 494
Mckenzie, A., 44, 55
Mackenzie, R. D., 492, 494
Mackie, F. P., 158, 159
Mackie, P., 87, 100
McKinley, E. B., 486, 487, 494
McKinney, R. A., 455, 462
McKuskey, J. M., 259
McLachlan, A. E. W., 258, 260
McLachlan, E. A., 344
McLachlan, W. W. G., 471, 473
MacLaughlin, J. H., 348, 359
Maclean, G., 157, 159, 172, 173
McLeod, M., 383, 390, 420
McMahon, B. T., 293, 295
McMullen, J., 244
McNamara, P. J., 431
Madajewa, O. S., 117
Maderna, C., 257, 260
Magendie, 60‘ 64
Magidson, O. J., 114, 117, 137, 147
Mahony, J. F., 285, 286
Maier, N. R. F., 425
Maier-Bode, H., 274, 469
Maigre, E., 294, 296
Manganey, L., 448, 449
Manin, Y., 213, 217, 286
Manson, D., 8, 9, 33, 34, 149
Manson-Bahr, P. H., 65, 67, 71, 75
Maplestone, P. A., 2, 6, 13, 14, 15, 23, 27, 30, 32
Maranon, J., 125, 126
Maraun, L., 351, 359, 415, 418
Marcenac, M., 40, 41
Marchal, J. E., 241, 244
Marchoux, E., 78, 84, 316, 317, 319
Marcus, M., 410, 411, 412
Marcus, P. M., 489, 493
Maria, G., 28, 30
Marino, P., 128, 131
Marino-Asserto, P., 129
Markianos, J., 317, 319
Marshall, E. K., 330, 332, 333, 334, 336, 337, 338, 340, 341, 342, 343, 344, 426, 428, 436, 437, 444, 445, 448
Marshall, J., 18, 22
Marshall, J. W., 266, 268
Martenstein, H., 239, 244
Martin, R. R., 61, 64
Martindale, W. H., 65, 68
Marty, J., 157, 158
Marven, H. P., 402, 409
Massa, M., 139, 147
Massell, B. F., 352, 359, 432, 435
Massias, C., 133, 136
Massingham, R., 354, 359
Masson, L. V., 253, 260
Masuzawa, T., 309, 312
Mattei, C., 61, 64
Mattice, M. R., 258, 260
Mauer, G., 492, 494
Mauer, M. E., 287, 296
Mauss, H., 136, 137, 147
Mayer, C., 293, 296
Mayer, K. F., 360
Mayer, M., 129, 131, 206, 209
Mayer, R., 453, 462
Mayer, R. L., 323, 329, 338, 341, 363, 369, 381, 382
Mayer, S., 389
Mayne, F. S., 104
Maxon, H., 72, 75
Mazza, S., 103, 104, 178, 179
Meck, J. S., 486, 494
Meissner, W., 415, 418
Mellon, R. R., 332, 336, 360, 383, 387, 390, 391, 409, 412, 417, 419, 443, 455, 459, 462
Melton, G., 476, 479
Mendelsohn, W., 278
Menk, W., 71, 75
Menkin, V., 294, 296
Menville, J. G., 433, 435
Mercader, N., 294, 296
Merriman, K. S., 268
Mertins, P. S., 388, 391
Mertins, P. S., Jr., 388, 391
Mesnil, F., 192, 198
Metzger, J. E., 8, 9
Meunier, P., 346, 359
Meyer, F., 457, 463
Meyer, J., 395
Meyer-Heine, A., 350, 359
Meyer zu Hörste, G., 351, 359
Mhaskar, K. S., 10, 12, 25, 26, 27, 30
Michie, A. M., 405, 409
Mickle, F. L., 460, 463
Miégeville, J., 424, 425
Mietzsch, F., 136, 137, 147, 322, 324, 329
Milian, G., 249, 253, 260
Millar, J. H. D., 350, 359
Millett, J., 332, 336, 355, 359, 387, 388, 391, 426, 428
Millous, 173
Mills, C. H., 244
Milne, J. C., 25, 27
Minec, 318, 319
Minkenof, J. E., 350, 359
Minot, A. S., 7
Miskjian, H. G., 259
Missiroli, A., 109, 117, 128, 131
Mitchell, A. G., 360
Mitchell, R. H., 402, 407
Model, A., 438, 400
Moen, J. R., 360
Moetsch, J. C., 341, 382
Moir, K. T., 153, 154
Mollgard, H., 290, 292, 296
Mollgaard, H., 288, 296
Molloy, D. M., 13, 14, 15, 18, 20, 23, 25, 26, 27
Moncorps, C., 215, 217
Monier, H. M., 133, 134, 136
Mönnig, H. O., 39, 41
Montel, G., 318, 319, 320, 321
Montel, L. R., 490, 494
Montel, R., 316, 317, 318, 319, 320, 321
Montestnic, E., 46, 48, 55
Montezin, G., 188, 196
Montgomerie, R. F., 413, 414, 489, 493
Moore, C., 424, 425
Moore, H. F., 470, 473
Moore, J. E., 244, 271, 285, 286
Moreau, P., 121, 124
Morgan, G. T., 160, 161, 162, 181, 182, 197
Morgan, W. A., 360
Morgenroth, J., 322, 329, 465, 468, 470, 473
Morrell, C. A., 242, 243, 244
Mosna, E., 109, 117, 128, 131, 144, 146, 147
Motz, C., 448, 449
Mouat, F. J., 299, 302
Moulinard, M., 46, 55
Mu, J. W., 483
Mudd, S., 481, 482
Muether, R. O., 345, 359
Mühlens, P., 71, 75, 88, 108, 123, 127, 131, 135, 136, 138, 148
Muir, E., 87, 100, 298, 302, 309, 312, 315, 319, 320, 321
Mukerji, A. K., 2, 6, 13, 14, 15, 23, 27, 30, 32
Mukerji, B., 8, 9
Mukherjee, B., 59, 63
Mulcahy, T. M., 441. 443
Mull, J. W., 445, 448
Mullick, M. N., 91, 99
Mulzer, P., 90, 101, 239, 244, 280, 283
Murray, D. H., 255, 261
Murgatroyd, F., 161, 162, 165, 166, 188, 189, 191, 192, 193, 198, 199, 212, 213, 215, 217, 218, 219, 222, 230, 231, 273, 274
Myers, C. N., 221, 297
Myers, G. B., 433, 435

NADER, A., 418, 419
Nag, S. C., 28, 30
Naito, T., 188, 198
Nandi, B. K., 129, 131
Napier, L. E., 66, 67, 90, 91, 94, 95, 96, 97, 98, 99, 100, 101, 104
Natwig, H., 421
Navarro-Martin, A., 84, 159, 160, 286
Nazmi, M., 47, 55
Neal, J. B., 355, 359, 388, 391
Neale, R. C., 29, 30
Necheles, H., 489, 494
Nelson, W. L., 473

Neogi, S. K., 90, 100
Neter, E., 394, 396
Neumann, C. Z., 413, 414
Neumann, H, 198
Newman, B. A., 434, 435
Newman, B. M., 151
Newman, R. E. U., 78, 84
Nguyen-Van-Tho, 490, 494
Nhuan, N. N., 318, 319
Nicholson, W. F., 356, 359
Nicol, W. D., 130, 131
Nicolas, C., 316, 319
Nicolas, L., 134, 135
Nicolau, S., 204, 209
Nicolle, P., 205, 208
Nierenstein, M., 192, 196
Nitti, F., 323, 329, 346, 359, 381, 382, 391, 403, 407, 410, 412, 425, 449, 454
Niven, 140
Nocht, B., 129, 131, 153, 154
Noguchi, H., 98, 100
Nolasco, J. O., 305, 307, 308
Northey, E. H., 488, 494
Nossina, V., 68, 70

OAKLEY, C. L., 340, 341, 487, 494
Obermayer, M. E., 271
O'Connell, J. T., 439, 440
Oesterlin, M., 51, 55, 109, 111, 116, 117, 130, 131
Offant, 292, 295
O'Hanlon, O. J., 400, 401, 402, 409
Oka, S., 188, 198
O'Leary, P., 244, 252, 261
Olitsky, P. K., 492, 494
Oliver, W. A., 388, 392, 456, 463
O'Malley, C. K., 405, 409
O'Meara, R. A. Q., 453, 455, 461
Orenstein, J. A., 122, 123
Orr, H., 261, 400, 409
Oser, B. L., 261
Osgood, E. E., 384, 391, 436, 437, 455, 457, 458, 463
Osterberg, A. E., 79, 84, 333, 335, 417, 418, 474, 477, 478, 479
Osterholz, E., 385, 391
Oto, M., 309, 312
Ott, E., 341, 381, 390, 396, 412
Ottenberg, R., 418, 419, 445, 448
Otto, R., 480, 482
Oxer, D. T., 28, 30

PACCLEA, B. L., 410, 412
Page, W. A., 413, 414
Paget, H., 8, 9, 302, 306, 308, 312
Paillard, H., 255, 259
Paine, C. G., 436, 437, 454, 459, 461
Paladino-Blandini, A., 129, 131
Palazzoli, M., 403, 448, 449
Paldrock, A., 320, 321
Palmer, R. B., 271
Palmer, W. H., 354, 359
Pampana, E., 125, 126
Panayotatou, A., 46, 55, 71, 75, 88
Papamarku, P., 216, 217, 277, 278
Parish, H. J., 295, 383, 390, 420, 422
Parran, T., 244
Parrot, L., 122, 123
Parsons, R. P., 266, 268
Parvulescu, 125, 126
Pascal, I., 357
Pasco, A. M., 13, 15, 24
Paton, J. P. L., 446, 448
Patrick, L. B., 436, 437, 454, 459, 461
Patterson, H. A., 290, 296
Pauget, P., 290, 296
Paul, B. M., 60, 64
Paul, S. N., 96, 99
Paul Raj, M., 321
Payne, F., 16, 22
Payne, G. C., 16, 22, 26, 27
Payre-Ficot, L., 289, 296
Peacock, D., 76, 77
Pedlow, J. T., 195, 198
Peebles, L. M., 346, 358
Peel, E., 324, 235
Peirier, 302
Pelletier, 60, 64, 107, 117
Pepper, D. S., 400, 403
Perez, A., 125, 126
Peristeris, M., 140, 143, 147
Perkins, G. A., 309, 311, 312
Perkins, N. H., 353, 359
Perley, A, M., 334, 335, 388, 389, 424, 425, 444
Permar, H. H., 471, 473
Pernice, W., 415, 419
Perroncito, E., 2, 3, 6
Peter, F. M., 47, 55, 85, 86, 87, 100, 138, 148, 490, 494
Peters, B. A., 293, 296, 354, 359, 446, 448
Peters, R. J., 251, 257, 261
Peterson, S. C., 266, 268
Pfeiler, W., 206, 209
Phelps, J. R., 246, 261
Photinos, G. T., 102, 104
Pieri, J., 46, 55
Pierson, M., 268
Pigeaud, H., 348, 359
Pillai, K. V., 60, 63
Pinner, T., 293, 295
Pinto, J. O., 152
Pirot, 33, 34
Platou, E. S., 354, 360
Platt, R., 475, 479
Plimmer, H. G., 167, 169
Plumer, H. E., 438, 440
Poindexter, H. A., 183, 186
Pongratz, R., 393, 396
Pons, C., 235
Popescu, F., 56, 57
Portugal, H., 304, 307, 310, 312
Pottier, R., 156, 157, 159
Poulain, P., 325, 329
Povitsky, O. R., 423, 425
Power, F. B., 301, 302
Prain, D., 299, 302
Prieur, M., 156, 158, 205, 208
Probey, T. F., 243, 244, 264, 268
Proom, H., 332, 336, 343, 344, 392, 396
Puig, R., 465, 468
Purdie, A. W., 346, 347, 348, 357, 359, 410, 412, 445, 447, 448
Püschel, E., 351, 359
Pützer, B., 103, 104
Pyman, F. L., 18, 22, 60, 63, 69, 70, 74, 75, 77, 480, 482

QUASTEL, H., 160, 162, 220, 221, 222, 225, 227
Query, R. Z., 388, 391

RABE, P., 108, 117
Rabut, R., 286
Radvan, I., 122, 123
Ragiot, C., 121, 124
Ragu, J., 309, 312
Rai Chaudhuri, M. N., 95, 100
Raingeard, 157, 159
Raiziss, G. W., 268, 272, 338, 341, 368, 382, 479, 482
Rajam, R. V., 250, 251, 261
Rambo, V. C., 332, 336, 340, 341
Ramijean, R., 286
Rao, G. R., 306, 307, 308, 315, 319
Ratish, H. D., 333, 336
Rauh, A. E., 390
Rauschkolb, J. R., 259
Ravaut, P., 258, 261
Rawson, G. W., 33, 34
Ray, A., 380, 381, 408
Raybaud, A., 93, 100
Raynaud, 316, 319
Read, B. E., 303, 304, 308
Read, H., 152
Rebaudi, U., 257, 261
Recknagel, K., 353, 360
Reed, A. C., 65, 68, 73, 74, 78, 81, 82, 83, 85
Regendanz, P., 191, 198
Reichenow, E., 191, 198
Reid, G. C. K., 389, 391
Reid, E. E., 377, 382
Reimann, H. A., 477, 479
Rein, C. H., 281, 283
Reiner, L., 193, 195, 198, 223, 224, 327
Reinié, L., 422, 457, 459, 462
Renfrew, A. G., 473
Renrie, J. L., 351, 360
Rerebak, J., 369, 381, 468, 469
Reuter, F. A., 409, 412, 417, 419
Reynier, P., 294, 296
Rhinehart, J. F., 62, 64
Rhoads, C. P., 26, 27
Rhodes, A. J., 486 487, 494
Rice, J. B., 130, 131
Rich, A. R., 295, 296
Richard, G., 380, 381, 408
Richards, H. R. M., 255, 261
Richardson, A. P., 280, 282
Richardson, B. W., 21, 23

Richardson, L. A., 413, 414
Richter, R., 219, 222, 264
Riehl, G. Jr., 264
Rimington, C., 340, 341, 433, 434, 435
Robb, J. V. M., 181, 182, 197
Robbins, B. H., 7, 9, 13, 19, 23
Robert, A. E., 279, 283
Roberts, F. H. S., 3, 4, 6
Robertson, J., 281, 282
Robertson, K., 389, 391
Robertson, M., 191, 198
Robinson, H. E., 201, 202
Robinson, H. M., 256, 261, 271
Robinson, R., 132, 135
Robinson, W., 484, 485
Rochedix, J., 413, 414
Rodhain, J., 118, 119, 152, 168, 170, 234, 235
Roe, H. E., 85, 86
Roehl, W., 93, 100, 110, 117, 127, 131, 194, 197, 198, 214, 218
Roger, H., 292, 296
Rogers, L., 60, 64, 87, 106, 288, 296
Römer, H., 352, 353, 358
Ronse, M., 487, 494
Röper, E., 361, 362
Rosahn, P. D., 281, 283
Rosenberg, E., 200, 203, 468
Rosenheim, M. C., 475, 476, 479
Rosenmund, K. W., 11, 12
Rosenthal, S. M., 243, 244, 264, 268, 341, 368, 372, 273, 378, 380, 382, 383, 384, 385, 389, 390, 391, 392, 393, 394, 396, 397, 452, 463, 486, 487, 494
Ross, A. O. F., 162, 273, 274
Ross, I. C., 3, 4, 6
Ross, M. A., 495
Rossiansky, N., 240, 244
Rothe, O., 301, 302
Rothermundt, M., 219, 222, 263, 264
Rotter, H., 440, 441
Roubaud, E., 234, 235
Rouit, 292, 295
Rourke, G. M., 330, 336, 341
Rousset, J., 33, 34
Row, R. S. T. M., 303, 307
Rowntree, L. G., 64, 253, 261
Roy, A. C., 60, 63, 98, 99, 120, 123, 139, 140, 141, 146, 151
Rozen, E. A., 61, 64
Rubzow, M. W., 117
Rubinstein, B. N., 137, 148, 149
Rubinstein, P. L., 232, 233
Ruch, R. O., 259
Ruedemann, R., 250, 251, 262
Ruge, H., 250, 261
Rugna, I., 126
Rule, A. M., 281, 284, 469
Russell, A. J. H., 125, 126
Russell, D. S., 249, 261
Russell, H. G., 219, 222
Russell, P. F., 125, 126
Russu, V., 294, 296
Ryles, C. S., 315, 319
Ryrie, G. A., 314, 316, 319

SABIN, A. B., 492, 493, 494
Sager, R. V., 253, 261
Saha, B., 88, 100
Sako, N., 354, 360
Salah, M., 49, 55
Salaman, R. N., 202, 203
Salle, A. J., 480, 482
Salvin, M., 433, 435, 436
Sami, A., 348, 358
Sampson, W. L., 242, 244
Sams, W. M., 253, 262
Samson, J. G., 308, 310, 312
Sanchis-Bayarri, V., 286
Sanders, I. P., 121, 124
Santiago, S., 309, 312
Santillan, P., 309, 312
Santos, I., 309, 313
Santos, G. O., 125, 126
Saphirstein, H., 433, 435, 448, 449
Sardon, 46, 55
Sarrouy, C., 61, 64
Sato, S., 309, 312
Saunders, G. M., 281, 283
Sautet, J., 119, 134, 136
Sauton, 279
Savignoni, F., 28, 30
Sazerac, R., 279, 280, 283, 285, 286
Schaaf, F., 248, 256, 260
Schaffer, C. W., 370, 382
Schaffer, J. M., 35, 41, 42
Schamberg, J. F., 243, 244, 479, 482
Schapiro, D., 11, 12
Schechter, A. J., 141, 143, 148
Scheff, R. N., 202, 203
Scherber, G., 405, 409
Scherer, J. H., 29, 30
Schern, K., 183, 186, 198
Scheuble, R., 35, 41
Scheurer, O., 356, 360
Schilling, G., 188, 198
Schlingman, A. S., 32, 34
Schlossberger, H., 170, 174, 187, 190, 198, 232, 233, 274, 275, 276
Schmidt, E. G., 343, 344
Schmidt, H., 47, 55, 56, 87, 88, 92, 99, 100, 106, 117, 490, 494
Schmidt, J., 419, 420
Schmidt, L. E., 266, 267, 268
Schmidt, L. H., 392
Schneider, M., 408
Schnitzer, R., 180, 181, 200, 203, 468
Schnohr, E., 476, 479
Schoen, R., 286
Scholzke, K., 404, 405, 409
Schonberg, I. L., 433, 435
Schonhöfer, F., 127, 131
Schranz, H., 349, 360
Schreck, H., 198
Schreiner, K., 256, 261
Schreus, H. T., 431
Schröder, G., 289, 296
Schubert, M., 405, 409, 447, 448
Schüffner, R., 187, 190, 198, 274, 275
Schüffner, W., 2, 6
Schujman, J., 314, 318
Schulemann, W., 126, 127, 131
Schultz, E. W., 492, 494, 495
Schultze, A., 108, 117
Schulze, V. E., 124
Schwartz, A., 286
Schwartz, W. F., 438, 441
Schwentker, F. F., 355, 360, 393, 397, 428, 432, 435
Schwetz, J., 318, 319
Scorgie, N. J., 361, 362
Scudi, J. V., 333, 336
Seastone, C. V., 345, 360
Secher, K., 291, 296
Seddon, H. R., 3, 4, 6
Selinger, E., 492, 495
Sellars, A. H., 495
Sen, B., 62, 63, 66, 67, 81, 82, 84, 120, 123, 141, 146
Sen, S., 66, 67, 81, 82, 84
Serefis, S., 280, 283
Sergent, A., 134, 135, 136
Sergent, E., 134, 136
Serra, D., 118, 119
Severac, M., 264, 268, 272, 382, 479, 482
Shaffer, L. W., 257, 258, 261
Sharlit, H., 434, 435
Sharp, T. M., 320, 321
Shaw, H. A., 271
Sherman, W. L., 268
Shillinger, J. E., 31, 34, 35, 41
Shinn, L. E., 360, 412, 417, 419, 442, 443
Shipp, M. E., 484, 485
Shivers, G., 246, 261
Shohl, A. T., 477, 479
Shonle, H. A., 271
Short, C. L., 293, 296, 440
Shotten, C., 475, 479
Shropshear, G., 491, 495
Shute, P. G., 130, 131
Sicault, G., 134, 136
Sicé, A., 159, 160, 220, 222
Sichet, P., 294, 296
Sigel, O., 352, 360
Silberstein, W., 180, 181, 200, 203
Silkergleit, H., 252, 261
Simić, T. V., 215, 216, 218, 277, 278
Simpson, C. K., 254, 260
Sincke, G. E., 102, 105
Singer, E., 116, 146, 147, 194, 195, 197, 198, 218, 222, 223, 226, 227
Singer, K., 442, 443
Sinton, J. A., 110, 117
Sioli, F., 127, 131, 135, 136, 138, 148
Slanetz, C. A., 370, 380, 489, 493
Slatineau, A., 125, 126
Sloan, L. L., 156, 159
Slosse, M., 255, 261
Smith, A., 349, 353, 360
Smith, D. E., 481, 482
Smith, F. W., 346, 360
Smith, H. L., 487, 495

Smith, H. W., 211, 218, 277, 278
Smith, J. T., 445, 448
Smith, S., 380, 390, 412, 483
Smithburn, K. C., 295, 296
Smyth, H. F., 370, 382
Smythe, C. V., 324, 227
Snodgrass, W. R., 251, 257, 261, 350, 351, 360
Soca, F., 62, 64
Soedjono, D. P., 439, 441
Soehring, K., 460, 463
Soesilo, R., 145, 148
Soffer, L. J., 251, 252, 259, 261
Solomon, W., 108, 116, 117, 118
Sonnenberg, E., 285, 286
Soper, F. L., 28, 30
Sorinson, N. S., 102, 105
Sorley, J. T., 315, 319
Southwell-Sander, G., 394, 396, 439, 440, 442
Southworth, H., 443, 444
Spaar, A. E., 142, 147
Spaulding, J. B., 279, 280, 283
Spillman, A., 257, 262
Spray, R. S., 421
Stableforth, A. W., 361, 362
Stanley, W. M., 311, 313
Steenken, W., 287, 296
Steiner, G., 287, 290, 296
Steiner, M., 286, 297
Stephani, T., 294, 297
Stephenson, C. S., 246, 262
Stephenson, D., 323, 328, 345, 364, 366, 367, 369, 380, 381, 383, 390, 420, 422, 452, 461
Steppuhn, 171, 174
Stern, R. L., 413, 414
Stevenson, H. A., 480, 482
Stewart, J. D., 330, 336, 340, 341
Stewart, J. L., 177, 179
Stewart, M., 31, 34
Steyn, D. G., 315, 319
St. John, J. H., 66, 68, 70
Stokes, J. H., 239, 240, 244, 249, 250, 251, 162, 269, 276
Stoll, N. R., 2, 6
Stone, C. T., 121, 124
Storm, C. J., 129, 131, 138, 139, 146, 148
Stoughton, R. W., 32
Strangeways, W. I., 163, 164, 165, 166, 167, 214, 215, 217, 218, 221, 224
Strauss, M. B., 443, 444
Strickland, C., 151
Strukow, I. T., 114, 117
Struthers, E. B., 88, 94, 95, 100
Stühmer, 219, 222
Stümpke, G., 448, 449
Subbarao, K. V., 419, 420
Suchier, W., 413, 414
Sulkowitch, H. W., 424, 425
Sulzberger, M. B., 248, 261, 281, 283
Summerfeldt, P., 477, 479
Sur, S. N., 90, 100
Surerus, D., 301, 302
Sweet, L. A., 273
Swellengrebel, N. H., 128, 131
Swift, H. F., 247, 262, 352, 360
Symmers, W. S. C., 484, 485

TAHA, S., 74
Tanguy, Y., 413, 415
Tara, S., 119
Tarejew, E., 134, 136
Tate, P., 128, 131, 132, 136
Tatum, A. L., 227, 228, 229, 264, 273
Tatum, W. B., 469
Taylor, G. C., 266, 267, 268
Taylor, H. M., 141, 143
Taylor, H. W., 424, 425
Taylor, I., 350, 357
Taylor, J., 242, 244
Teggart, B., 424, 425
Tegner, W., 290, 295
Tejera, E., 206, 209
Telling, M., 388, 392, 456, 463
Temming, H., 415, 419
Tenney, A. C., 74, 75
Teplitz, M. M., 287, 397
Terrill, F. I., 293, 297
Terry, B. T., 213, 218
Tettweiler, J., 10, 12
Thayer, J. D., 409
Thévenet, V., 413, 415
Thiel, H., 292, 295
Thimme, B., 352, 353, 358
Thompson, A. R., 492, 495
Thompson, H. G., 49, 55
Thompson, J. D., 167, 169
Thompson, J. W., 218, 222
Thonnard-Neumann, E., 139, 148
Thron, H., 112, 116
Throne, B., 290, 297
Thung, T. H., 201, 203
Tietze, A., 431, 432
Tiffeneau, 385
Tiling, B., 356, 360
Tillett, W. S., 455, 463
Tisdall, F. F., 493, 495
Tixier, L., 356, 360
Tomb, J. W., 28,30, 308, 313
Tonndorff, H., 350, 360
Touraine, A., 439, 441
Tournade, A., 61, 64
Tournier, 88, 100
Townsend, T. M., 441, 443
Tran-Tan-Phat, 286
Traschsler, W. H., 355, 360
Trefouël, J., 84, 136, 159, 160, 163, 323, 329, 381, 391, 403, 407, 425
Trefouël, Madame, 84, 136, 159, 160, 163, 323, 329, 381, 391, 403, 407, 425
Trensz, F., 134, 136
Trevan, J. C., 99, 111, 116, 308, 309, 312, 337, 341
Trillat, A., 173, 174
Tropp, C., 116, 139, 141, 148
Trumper, A., 440, 441
Tsykalas, D., 51, 56
Tubangui, M. A., 13, 15, 24
Tudhope, C. R., 64
Tunnicliff, E. A., 32, 34
Türk, H., 415, 419

UDALAGAMA, 142
Ueno, S., 320, 321
Uhlenhuth, H., 47, 56, 90, 101
Uhlenhuth, P., 238, 244
Underwood, P. C., 15, 24, 42, 56, 57
Unger, F., 460, 463
Unshelm, E., 433, 435, 460, 463
Ury, J., 320, 321
Usilton, L. J., 240, 244
Utkina-Ljukowzewa, 171, 174

VAISMAN, A., 285, 286, 323, 329, 373, 375, 378, 381, 391, 392, 396, 399, 406, 408, 449, 455, 456, 457, 458, 462, 464, 465, 483, 486, 490, 494
Valery-Radot, P., 290, 297
Vamos, L., 286
Vamos, S., 158, 159
Van den Branden, F., 156, 157, 159, 235
Van Heukelom, A. S., 143, 148
Van Hoof, L., 169, 170, 174, 234, 235
Van Nitsen, R., 50, 52, 56, 118, 119
Van Putte, P. J., 264
Van Rooyen, C. E., 486, 487, 494
Van Slyke, C. J., 401, 409
Van Slype, W., 143, 148
Vardy, E. C., 143. 148
Varma, R. L., 103, 105
Vaucel, 159, 160
Vedder, E. B., 64
Velu, H., 424, 425
Verghese, M. C., 293, 295
Verhoog, M. J., 166, 167, 289, 295, 464
Verwoort, H., 2, 6
Vianna, G., 87, 101
Vigne, P., 102, 105
Vildé, L., 402, 407
Villani, A. J., 416, 419
Villaret, M., 257, 262
Vincent, M., 128, 131, 132, 136
Vitenson, I., 355, 360
Voegtlin, C., 211, 214, 218, 222, 223, 227, 264, 268, 277, 278
Vogt, P., 134, 135, 136
Volger, G., 108, 117
Vonder Heide, E. C., 435
Von Haebler, T., 418
Von Issekutz, B., 226, 227
Von Jancsó, H., 183, 186, 189, 198, 199, 201, 202, 203, 216, 226, 227, 230, 232, 233
Von Jancsó, N., 183, 186,189, 195, 198, 199, 200, 201, 202, 203, 216, 226 227, 230, 232, 233

Von Oettingen, W. F., 11, 12, 429, 430
Von Schürer, F., 177, 179
Von Unruh, V., 8, 9
Vuilliemoz, 251, 259

WAGNER, O., 85, 86
Wagner-Jauregg, T., 316, 318
Walbum, L. E., 294, 297
Waldron, W. R., 377, 382
Walker, O., 420
Walls, L. P., 181, 182, 197
Walsh, D. F., 406, 409
Walsh, J. H. T., 60, 64
Walters, A. L., 64
Walton, E., 10, 12, 160, 161, 162
Wampler, F. J., 130, 131
Wang, K. C., 13, 24
Ward, C. B., 13, 14, 15, 16, 17, 20, 23, 24, 456
Ward, R. O., 45, 54
Warma, J. D., 103, 105
Wats, R. C., 141, 148, 153, 154
Webster, M. H., 405, 409
Wechselmann, W., 238, 244
Wedekin, E., 10, 12
Weed, L. A., 480, 482
Weese, H., 92, 101
Weiland, G. S., 8, 9
Weinberg, M. H., 355, 360, 442, 443
Weinberg, T., 247, 262
Weise, W., 116, 139, 141, 148
Weiser, A., 415, 419
Weisman, D., 258, 259
Welch, H., 460, 463
Wellcome, 107, 118
Wells, H. G., 287, 296
Wendel, W. B., 447, 448
Wengatz, H. F., 398, 409
Wentworth, J. A., 460, 463
Werbitzki, F. W., 191, 199
Werner, S., 253, 262
West, A. P., 309, 312, 313
Westerlinck, H., 316, 318
Wever, G. K., 267, 268
Whicher, A., 361, 362, 487, 495
Whitby, L. E. H., 328, 329, 339, 341, 364, 382, 384, 386, 392, 393, 397, 456, 463, 469
White, A. C., 75, 77
White, C. F., 244
White, E. C., 479, 482
Whitmore, E. R., 151
Whitney, L. F., 489, 493
Wieder, L. M., 266, 268
Wien, R., 242, 243, 245, 386, 392
Wigdor, M., 13, 22
Wigmore, J. B. A., 88, 101
Wijerama, E. M., 143, 147
Wilcox, D. A., 359, 396, 425
Wilcox, W., 250, 262
Wile, U. J., 244, 251, 253, 262
Wiley, C. J., 43, 56
Wilkinson, W. E., 402, 409
Williams, B., 348, 360
Williams, R., 389
Williamson, G. S., 259
Willien, L, J., 394, 397
Willis, T., 402, 409
Wingler, A., 127, 131
Wingo, E. H., 246, 259
Wishengrad, M., 423
Wittmann, J., 371, 372, 381
Witts, L. J., 291, 297
Wolf, C. H., 361, 362
Wolff, E., 72, 75
Wolinetz, 395
Wood, W. A., 9
Woods, A. C., 156, 159
Wooley, J. G., 487, 494
Wormall, A., 171, 173, 204, 208, 221
Wrenshall, R., 301, 302
Wright, F. H., 359, 396
Wright, H. N., 201, 203
Wright, W. H., 15, 24, 35, 41, 42, 56, 57

XALABARDER, C., 294, 297

YAMANOUCHI, T., 212, 217
Yanagisawa, K., 294, 297
Yates, T. M., 101
Yorke, W., 161, 162, 183, 186, 188, 189, 190, 191, 192, 193, 195, 198, 199, 212, 213, 215, 216, 217, 218, 219, 222, 230, 231, 273, 274
Youman, G. P., 398, 408
Young, C. J., 438, 441
Young, F., 388, 392
Young, R. H., 424, 425
Young, W. A., 64
Yutuc, L. M., 24

ZAHRA-NEUMANN, C., 97, 101
Zendel, D., 395, 397
Zottner, G., 424, 425
Zschucke, J., 173, 174
Zuccardi, E., 178, 179
Zürn, D., 404, 405, 409

SUBJECT INDEX

- **Acetarsol** (Acetylaminohydroxyphenylarsonic acid) in amœbiasis, 77
- **3(Acetylamino)-9(*p*-acetylaminophenyl)-10-methylphenanthridinium** chloride in trypanosomiasis, 182
- *p*-**Acetylaminobenzenesulphonamide,** 333
  - excretion, 333
  - relation to sulphonamide, 333
  - toxicity, 338
- *p*-**Acetylaminobenzenesulphinic** acid and streptococcal infections, 482
- **Acetylaminohydroxyphenylarsonic acid** (= acetarsol) in amœbiasis, 77
- *α*-**Acetylaminonaphthalene-4-sulphonamide** in streptococcal infections, 365
- **2(*p*-Acetoaminostyryl)-6 (*p*-aminobenzoylamino) quinoline** methoacetate in trypanosomiasis, 176
- **Acidosis** and sulphanilamide, 420
- **Acridine** derivatives in bacterial infections, 465
  - in malaria, 148
  - in trypanosomiasis, 180
- **Acriflavine** and bovine mastitis, 361
  - and drug resistance, 194
  - and psittacosis, 492
- **Acriflavine-resistant** *Bodo*, 191
- **Actinomyces** and alkyldimethylbenzyl ammonium chlorides, 483
- **Actinomycosis** and sulphanilamide, 420
- **Adenitis** and sulphanilamide, 361
- **Adrenalin** in malaria, 122
- **Agranulocytosis** and bismuth, 282
  - and gold salts, 291
  - and sulphanilamide, 437
- **Aldarsone** and syphilis, 272
- **Aldehyde** (formol gel) test in kala azar, 96
- **Alepol** in leprosy, 308
  - toxic effects, 303
- **Alkali** reserves and sulphanilamide, 335
- **Alkresta** ipecac. in amœbiasis, 66
- **6-*n*-Alkyl-*m*-cresols** in ascariasis, 19
- **Alkyldimethylbenzyl** ammonium chlorides and actinomycosis, 483
- **Alkylene diamines** in trypanosomiasis, 185
- **Alkylharmols,** amœbicidal action, 69
- **Alkylhydroxybenzenes** in ascariasis, 17
- **Alkylquitenines** in bird malaria, 112
- **Allantoin** and ulcers, 484
- **Allochyrysine** in leprosy, 320
  - in tuberculosis, 289
- **Amibiarson** in amœbiasis, 82
- **7-Amino-9(*p*-aminophenyl)-10-methylphenanthridinium** chloride in trypanosomiasis, 182
- *m*-**Aminobenzenesulphonamide,** toxicity, 337
- *o*-**Aminobenzenesulphonamide,** toxicity, 337
- *p*-**Aminobenzenesulphonamide.** *See* Sulphanilamide.
- **2(*p*-Aminobenzenesulphonamido) pyridine.** *See* M. & B., 693.
- *p*-**Aminobenzenesulphonamidedimethylamide.** *See* Uleron.
- *p*-**Aminobenzenesulphonanilide,** 365
- *m*-**Aminobenzenesulphonic** acid in streptococcal infections, 365
- **3-Amino-4-hydroxyphenylarsenious** oxide (Mapharsen) in syphilis, 264
- *p*-**Aminophenylstibonic** acid in kala azar, 89
- **Aminophenylsulphones,** pharmacology. 378
- **4-Amino-quinoline** derivatives in trypanosomiasis, 175
- **Aminostiburea,** 89
  - in treatment of kala azar, 58
- **Amœbiasis,** acetarsol in, 77
  - alkresta ipecac. in, 66
  - and arsphenamines, 83
  - auremetine in, 65
  - bismuth subnitrate in, 85
  - carbamidophenylarsonic acid (carbarsone) in, 79
  - conessine in, 75

Amœbiasis diiodohydroxyquinoline (chiniofon) in, 74
  emetine, action of in, 68
    antimony iodide in, 65
    bismuthous iodide in, 65
    camphorsulphonate in, 65
    hydrochloride in, 64
    periodide in, 65
  formylaminohydroxyphenylarsonic (tréparsol) acid in, 79
  gavano in, 67
  hexylresorcinol in, 86
  iodaseptine cortial in, 85
  iodoform in, 85
  ipecacuanha alkaloids in, 58
  kurchi-bark in, 75
  quinoline derivatives in, 70
  rivanol in, 85
  vioform in, 72
  yatren in, 70
Amœbicidal action of benzylquitenine hydrochloride, 69
Amylene dichlorides in ankylostomiasis, 40
*iso***Amylhydrocupreine** (eukepin), 109
Anæmia and sulphanilamide, 441
β-**Angelicalactone** in ascariasis, 11
Aniline dyes and drug resistance, 187
    in leprosy, 314
Anil-quinoline derivatives in trypanosomiasis, 175
Ankylostomiasis and amylene dichloride, 43
  and carbon tetrachloride, 27
  and chloroform, 25
  and hexachloroethane, 34
  and hexylresorcinol, 15
  and oil of chenopodium, 25
  and pentachloroethane, 35
  and tetrachloroethane, 35
  and tetrachloroethylene, 30
  and thymol, 25
  treatment of, 25
Anthelmintics, action of, 5
  history of, 1
  methods of testing, 2
Anthiomaline in schistosomiasis, 46
Antimalarial drugs and infectivity of plasmodia, 150
Antimony, action on liver, 49
  aromatic compounds and drug resistance, 187
  in schistosomiasis, 43
    preparations, action on adrenals, 98
Antimony, action on bone-marrow, 98
    in trypanosomiasis, 187
  pyrocatechol compounds in schistosomiasis, 43
  quinquevalent compounds in kala azar, 89, 93, 96
    in oriental sore, 102
    method of action in kala azar, 97
    in dermal leishmaniasis, 107
    in muco-cutaneous leishmaniasis, 103
    toxic reaction, 97
Antimonyl tartrates in filariasis, 56
    in leishmaniasis, 87
    and leprosy reaction, 320
    in oriental sore, 101
    in *Parafilaria multipapillosa*, 57
    in schistosomiasis, 44
Antimosan in kala azar, 88, 99
  in schistosomiasis, 47
  in trypanosomiasis of cattle, 168
*Apo***quinine** and *apo*quinidine in bird malaria, 113
Appendicitis and sulphanilamide, 424
Aralkyl derivatives of 4 : 4′-diaminodiphenylsulphone, 377
Aromatic arsenicals, chemotherapeutic indices, 228
Arsenic, absorbed by normal trypanosomes, 193
    absorbed by resistant trypanosomes, 193
  aromatic compounds and drug resistance, 187
  brown, in trypanosomiasis, 167
  content of cerebrospinal fluid, 221
  yellow, in trypanosomiasis, 167
Arsenical compounds and trypanosome infections, specific relationship, 222
Arsenicals, quinquevalent, excretion, 218
    action on trypanosomes, 212
    in trypanosomiasis, 155
  tervalent, chemotherapeutic indices and resistant factor, 236
  ter- and quinquevalent, in syphilis, 277
Arsenobenzene compounds in syphilis, 238
Arseno-detoxin in trypanosomiasis, 166
Arsenophenylglycine and drug resistance, 190
Arsenopyridines in syphilis, 274
  in trypanosomiasis, 167

Arsenoxides, action on trypanosomes, 210
Arsonic acids, action on trypanosomes, 210
  containing amide-groups in trypanosomiasis, 166
Arsphenamine derivatives and hæmorrhagic encephalitis, 249
    and hepatitis, 249
    and medical shock, 246
    and sensitization, 247
    and toxicity, 246
  dermatitis, 247
  dermatitis and thiosulphate, 258
  preparations, therapeutic standardization, 240
  soluble preparation, 263
Arsphenamines, and amœbiasis, 83
  and " patch " test, 256
  in cerebrospinal fluid, 221
  reactions, prevention, 256
  solution and oxidation, 243
Arthritis and gold salts, 291
  and sulphanilamide, 361
Arylthioarsenites in trypanosomiasis, 163
Ascariasis and 6-*n*-alkyl-*m*-cresols, 19
  and alkylhydroxybenzenes, 17
  and *n*-butyl chloride, 39
  and cresyl butyrolactones, 11
  and hexylresorcinol, 12
  and santonin, 10
  and syntonin-a, 11
  treatment, 7
Ascaridole, 7
  toxicity, 8
Atebrin and blackwater fever, 152
  action on malaria parasites, 145
  chemical structure, 137
  estimation, 141
  in bird malaria, 138
  in human malaria, 143
  musonate, 138
  pharmacology, 138
  physical properties, 137
  prophylactic action, 145
  toxicity, 142
Atebrin-plasmoquine in malaria, 149
Atebrin-quinine in malaria, 149
Atoxyl, action on trypanosomes, 213
Auremetine in amœbiasis, 65
Aurodetoxin in bacterial infections, 464
  in *Spirochæta duttoni* infections, 464
  in syphilis, 464
  in tuberculosis, 289
Aurophos in tuberculosis, 289
Avenyl in leprosy, 328
Azo dyes, active component, 452

Bacteria, action of sulphonamide drugs on, 454
  and acridine derivatives, 465
  variation in susceptibility to sulphonamide drugs, 451
Bacterial capsules and sulphonamide drugs, 456
  endocarditis and sulphonamide drugs, 356
  infections, chemotherapy, 322
  toxins, neutralization by sulphonamide drugs, 457
Bactericidal action of sulphonamide drugs, 454
Bacteriostasis and sulphanilamide, 454
Bacterium aerogenes and sulphonamide drugs, 423
Bact. coli and hexylresorcinol, 13
    infections of urogenital tract and mandelic acid, 475
      and prontosil, 416
      and prontosil soluble, 416
      and sulphanilamide, 415
    proseptasine in, 418
    soluseptasine in, 418
    uleron in, 418
  liver abscess and sulphanilamide, 418
  meningitis and sulphanilamide, 418
Bact. typhosum and sulphanilamide, 419
Balanitis and sulphanilamide, 424
Bartonella infections and Sdt. 386 B., 483
Bayer 205. *See* Germanin.
Bayer 7602 (Ac) in American trypanosomiasis, 178
Bejel and bismuth, 281
Benzoylaminostyrylquinoline compound and trypanosomiasis prophylaxis, 207
Benzylquitenine hydrochloride as amœbicide, 69
Berberine and oriental sore, 102
Bilharziasis. *See* Schistosomiasis.
Bismuth and agranulocytosis, 282
  bejel, 281
  by mouth, 280
  in syphilis, 279
  preparations, electric charge, 279
  subnitrate in amœbiasis, 85
  toxic reactions, 281

Blackwater fever and atebrin, 153
  and plasmoquine, 153
  and quinine, 152
Blood pigments and sulphanilamide, 444
Bodo and resistance to acriflavine, 191
Boils and sulphanilamide, 411
Bone marrow and antimony, 98
  and sulphanilamide, 384
Bovine mastitis and acriflavine, 361
  and rivanol, 361
  and sulphanilamide, 361
Brilliant green in leprosy, 314
Brucella infections in animals and sulphonamide drugs, 415
  in man and sulphonamide drugs, 413
n-**Butyl** chloride in Ascariasis, 39
*iso***Butylhydrocupreine** in bird malaria, 109
n-**Butylidene** dichloride in *Strongylus* infections, 39

C77 in malaria, 109
Cadmium in tuberculosis, 294
Calcium mandelate in uro-genital infections, 476
  salts in tuberculosis, 287
Camphorsulphonate of emetine in amœbiasis, 65
Capsule formation in bacteria and sulphonamide compounds, 456
Carbarsone (4-carbamidophenylarsonic acid) in amœbiasis, 79
  action on *Trichomonas*, 83
  in giardiasis, 82
  toxic reactions, 82
  toxicity for animals, 80
Carbon tetrachloride in ankylostomiasis, 27
    toxic action on liver, 28
  trichloride in ankylostomiasis, 36
Carbuncles and sulphanilamide, 411
Cellulitis and sulphonamide compounds 351
Cephæline hydrochloride as amœbicide 68
Certuna in malaria, 135
Chancroid and sulphanilamide, 422
Chaulmoogra esters of amino-alcohols 309
  oil, 299
    in leprosy, 308
    local action, 305
    physical properties, 300
Chaulmoogra oil, toxic action, 303
  preparations in leprosy, 311
dl-**Chaulmoogric** acid, 311
Chaulmoogric acids, ethyl esters, 309
Chaulphosphate, 310
Chemical structure and therapeutic action of sulphonamide compounds, 451
Chemoprophylaxis of malaria with atebrin, 145
    with plasmoquine, 129
    with quinine, 122
  of puerperal fever with sulphonamide compounds, 348
  of scarlet fever with sulphonamide compounds, 354
  of syphilis, 285
  of trypanosomiasis with benzoylaminostyrylquinoline, 207
    with germanin 205
    with orsanine, 206
    with tryparsamide, 204
  of virus infections, 492
Chemotherapeutic interference, 200
    and accessory respiratory catalysts, 202
    and redox potentials, 201
    in vitro, 201
Chenopodium in ascariasis, 7
  oil, ascaridole content, 8
Chinofon in amœbiasis, 70
Chlorinated alkyl hydrocarbons in ankylostomiasis, 35
  butanes in cylicostomosis of horses, 40
Chloroform in ankylostomiasis, 35
2-Chloropentane in ankylostomiasis, 36
Cinchona alkaloids, constitution, 108
    history, 106
    in bird malaria, 110
    in human malaria, 120
    in stereochemistry, 109
  febrifuge, 124
Cinchonidine in bird malaria, 111
Cinchonine in bird malaria, 111
Cod liver oil in osteomyelitis, 484
    in ulcers, 484
Common cold and sulphanilamide, 487
Conessine in amœbiasis, 75
Copper preparations in leprosy, 320
    in schistosomiasis, 52
o-**Cresylbutyrolactone** in ascariasis, 11
Critical testing of anthelmintics, 3
Cyanosis and plasmoquine, 128
  and sulphonamide compounds, 444
Cysteine in leprosy, 320

DB 32 constitution, 368
DB 87 constitution, 368
  in gonorrhœa, 403
N-Decane-1 : 10-diamidine 2HCl in trypanosomiasis, 185
Dehydrochaulmoogric acid, constitution, 302
Demethoxyemetine as amœbicide, 68
Dermatitis and arsphenamines, 247
  and bismuth, 282
  and gold salts, 291
  and mapharsen, 267
  and neocryl, 274
  and sulphanilamide, 432
  and tartar emetic, 45
  and trisodarsen, 269
4 : 4′-Diacetylaminodiphenylsulphone (rodilone) (constitution), 328
  in gonorrhœa, 403
  in pneumococcal infections, 385
  in streptococcal infections, 372
  toxicity, 372
4:4′-Diacetyldihydroxydiphenylsulphone in streptococcal infections, 373, 393
4 : 4′-Diaminodiphenylsulphide in streptococcal infections, 378
4 : 4′-Diaminodiphenylsulphone,
  aralkyl derivatives, 377
  constitution, 371
  glucose, 377, 387
  in pneumococcal infections, 385
  in streptococcal infections, 375
  modification of molecule, 373
  modification of sulphone group, 375
  Schiff's bases, 376
  substitution in amino group, 376
  toxicity, 385
  unsymmetrical compounds of, 377
Diaminodiphenylsulphones, pharmacology, 378
Di-(β-amino-β-carboxyethyl)-benzamide-*p*-thioarsinite in trypanosomiasis, 165
Diarsonic acids in trypanosomiasis, 163
Dicuprène in schistosomiasis, 52
8-Diethylamino-*n*-prophylamino-quinoline (Fourneau 728) in bird malaria, 133
8-Diethylamino-undecylamino-6-methoxyquinoline (Fourneau 852) in bird malaria, 133
Diethylene glycol, toxicity, 429
Diglutathionyl-4-acetamido-2-hydroxyphenylthioarsinite in trypanosomiasis, 165
Dihydrocinchonidine, constitution, 109
  in bird malaria, 111
Dihydrocinchonine, constitution, 109
  in malaria, 111
Dihydrocupreidine ethers in bird malaria, 115
Dihydroquinamine in malaria, 119
Dihydroquinidine, constitution, 109
  in bird malaria, 111
Dihydroquinine, constitution, 109
  in bird malaria, 111
Diiodohydroxyquinoline in amœbiasis, 74
4 : 4′-Dinitrodiphenylsulphide in streptococcal infections, 378
4 : 4′-Dinitrodiphenylsulphone in streptococcal infections, 375
Diphenyl sulphones, 371
  sulphides, 371
Diphtheroids and sulphanilamide, 423
Dirofilaria immitis and antimony salts, 56
Dissociation constants of atebrin, 138
Distemper canine and sulphanilamide, 361
    and sodium sulphanilyl sulphanilate, 489
  feline and sulphanilamide, 361, 487
Disulphanilamide, 328, 367
  in meningococcal infections, 392
  in staphylococcal infections, 409
  in streptococcal infections, 367
Dn. 7 (stibilase) in schistosomiasis, 51
  in trypanosomiasis, 168
Dn. 9 in trypanosomiasis, 168
D. 12 in trypanosomiasis, 169
Dn. 18 (tristibine) in schistosomiasis, 51
  in trypanosomiasis, 169
N-Dodecane-1 : 12-diamidine 2HCl in trypanosomiasis, 185
Dog, puerperal infection and sulphanilamide, 361
Drug fever and sulphonamide compounds, 432
Drug resistance, 187
    classification, 190
    mechanism, 193
    rapidity of induction, 188
    stability, 191
    substances inducing, in trypanosomes, 187
    to germanin, 189
Drug-resistant syphilis, 283
Ducrey's Bacillus and sulphanilamide, 422

Emetamine, constitution, 59
  as amœbicide, 68
Emetine antimony iodide in amœbiasis, 65
  as amœbicide, 68
  bismuth iodide in amœbiasis, 65
  camphorsulphonate in amœbiasis, 65
  constitution, 59
  hydrochloride in amœbiasis, 64
  in oriental sore, 102
  in schistosomiasis, 51
  periodide in amœbiasis, 65
  pharmacology, 60
  toxicity, 61
Empyema and sulphonamide compounds, 355
Eosin in leprosy, 314
Epidermophyton inguinale and alkyl dimethylbenzylammonium chlorides, 483
Epileptiform convulsions and gold salts, 292
Erysipelas and sulphonamide drugs, 350
Ethanolamine mandelate in genito-urinary infections, 475
Ethyl*apo*cupreine and pneumonia, 472
Ethyl esters of chaulmoorgra, in leprosy, 308
    in toxicity, 305
Ethyldihydrocupreine (optochin) constitution, 109, 471
    in pneumococcal infections, 470
Ethyl-di-N-heptyl acetate, toxicity, 305
Ethylharmol as amœbicide, 69

Feline distemper and sulphanilamide, 361, 487
Filariasis and antimonyl tartrates, 56
  and fouadin, 56
Filarial lymphangitis and sulphanilamide, 57
Fluorescein in leprosy, 314
Formylaminohydroxyphenylarsonic acid (tréparsol) in amœbiasis, 79
Fouadin, concentrated, 50
  constitution, 47
  in schistosomiasis, 48
  toxicity, 49
Fourneau 309. *See* Germanin.
Fourneau 270. *See* Orsanine.
Fungi and alkyldimethylbenzyl ammonium chlorides, 483
  and neoarsphenamine, 483
Furuncles and sulphanilamide, 411

Gas gangrene and sulphonamide drugs, 420
Gavano in amœbiasis, 67
Germanin and carbohydrate metabolism, 226
  and reticulo-endothelial cells, 232
  and synergic action, 229
  constitution, 170
  estimation, 171
  in trypanosomiasis, 171
  prophylaxis of trypanosomiasis, 171
  toxic reactions, 171
Germanin-resistant trypanosomes, 189
Giardiasis and carbarsone, 82
Glanders and rubiazol, 424
Glucose derivative of 4 : 4′-diaminodiphenylsulphone in streptococcal infections, 387
    in lymphogranuloma inguinale, 491
Glutathione and action of arsenicals, 213
Gold dermatitis, 291
  preparations and sulphydryl group, 294
    in tuberculosis, 288
    in leprosy, 320
Gonococcal endotoxins and sulphonamide compounds, 406
  infections in mice and sulphonamide compounds, 406
    and hydroquinone, 483
  meningitis and sulphanilamide, 402
  ophthalmia neonatorum and sulphanilamide, 402
  rheumatism and sulphanilamide, 402
  vulvovaginitis and sulphanilamide, 401
Gonococci, action *in vitro* of sulphonamide compounds, 399
Gonorrhœa and DB 87, 403
  and M & B 693, 405
  and prontosil soluble, 401
  and rodilone, 403
  and soluseptasine, 403
  and sulphanilamide, 398
  and uleron, 403
Gorli seed oil, 302
Guanidine derivatives excretion, 221
    in trypanosomiasis, 183
    sugar metabolism, 183
Guanidine-resistant trypanosomes, 188
Gynocardia odorata seeds, 299

Hæmatopoietic system and sulphanilamide, 436

Hæmophilus influenzæ and
    sulphanilamide, 423
  meningitis and sulphanilamide, 424
Hæmophilus pertussis and
  sulphanilamide, 423
Harmaline hydrochloride as amœbicide, 69
Harmol as amœbicide, 69
o-n-**Heptylphenol** in ascariasis, 18
Heptyl resorcinol in ascariasis, 17
Herpes and prontosil, 486
Hexachloroethane in ascariasis, 35
n-**Hexyl** chloride in ascariasis, 36
6-*n*-Hexyl-*m*-cresol, 19
o-n-**Hexyl harmol** as amœbicide, 69
Hexyl resorcinol, action on *Ancylostoma duodenale*, 15
    on *Tænia saginata*, 15
    on *Trichuris*, 15
  and *Bacterium coli* infections, 13
  in ascariasis, 12
  toxicity, 16
Homologues of plasmoquin in bird malaria, 132
Horse, toxic reactions to sulphanilamide, 362
Hydroquinone and bacterial infections, 483
β-**Hydroxybutyric** acid in genito-urinary infections, 474
Hypersensitivity to arsphenamines, 247
  to sulphanilamide, 435

Immune bodies and chemotherapy of gonorrhœa, 401, 405
    of meningococcal meningitis, 393
    of pneumonia, 384
    of staphylococcal infections, 410
Impetigo contagiosa and sulphonamide compounds, 356
Infectivity of plasmodia and antimalarial drugs, 150
Influenza virus and sulphonamide compounds, 487
Iodaseptine cortial in amœbiasis, 85
Iodochlorohydroxyquinoline (vioform) as amœbicide, 72
Iodoform in amœbiasis, 85
Iodohydroxyquinolinesulphonic acid (chiniofon) in amœbiasis, 70

Jaundice and arsphenamines, 249
  and bismuth, 282
  and gold salts, 291
  and sulphonamide compounds, 448

K.324 in trypanosomiasis, 165
K.325 in trypanosomiasis, 165
Kala-azar and advantages of quinquevalent antimony, 96
  and aminostiburea, 89
  and antimonyl tartrates, 87
  and antimosan, 88
  and neostam, 89
  and neostibosan, 89
  and quinquevalent antimony compounds, 88
  and solustibosan, 92
  and stibacetin, 89
  and stibamine, 89
  and stibosan, 89
  and treatment by quinquevalent antimony, 93
  and urea stibamine, 89
  criteria of cure, 95
  mode of action of antimony compounds, 96
Ketogenic diet, 474
Kurchi in amœbiasis, 75
Kurchine in amœbiasis, 75
Kurchicine in amœbiasis, 75

Lambs, septicæmia and prontosil, 362
Leishmaniasis, cutaneous and mucocutaneous and quinquevalent antimony preparations, 101
  treatment. *See also* Kala-azar, 87
Leprosy and alepol, 308
  and aniline dyes, 314
  and avenyl, 320
  and brilliant green, 314
  and carbon dioxide snow, 320
  and chaulmoogra oil, 308
  and chaulphosphate, 308
  and cod liver oil, 306
  and cysteine, 320
  and ethyl esters of hydnocarpus oil, 309
  and eulykol, 310
  and fluorescein, 314
  and gold preparations, 320
  and mercurochrome, 320
  and methylene blue, 316
  and solganal-B oleosum, 320
  and trypan blue, 314
Lithium antimonyl thiomalate (anthiomaline) in schistosomiasis, 46
    in trypanosomiasis, 168
Ludwig's angina and sulphanilamide, 353

Lupus erythematosus and sulphonamide compounds, 356
Lymphadenitis of guinea-pigs and prontosil soluble, 361
Lymphocytic choriomeningitis and prontosil, 487
Lymphogranuloma inguinale and sulphonamide compounds, 490

Malaria and acridine derivatives, 148
  and alkylquitenines, 112
  and *apo*quinine and *apo*quinidine ethers, 113
  atebrin, 143
  atebrin-musonate, 138
  and atebrin-plasmoquine, 149
  and blackwater fever, 152
  and **C. 77,** 109
  and Certuna, 135
  and chemoprophylaxis by atebrin, 145
    by plasmoquine, 129
    by quinine, 122
  and cinchona alkaloids, 120
  and dihydrocupreine ethers, 115
  and dihydroquinamine, 119
  and 6-methoxyquinolines, 114
  and methylene blue, 126
  and mode of action of cinchona alkaloids, 115
  and N-undecamine 1 : 11 diamidine 2HCl, 186
  and oxyquinoline derivatives, 118
  and plasmoquine, 126
  and pregnancy, 130
  and quinine-atebrin, 149
  and quinine-plasmoquine, 149
  and sulphanilamide, 152
  and totaquina, 124
  avian and cinchona alkaloids, 100
    and homologues of plasmoquine, 133
  chemotherapy and the reticulo-endothelial system, 116
  in *Padda oryzivora*, 114
  of monkeys and sulphonamide compounds, 157
Malarial parasites, action of atebrin, 145
    of plasmoquine, 130
  infectivity and anti-malarial drugs, 150
Mandelic acid and sulphanilamide compared, 417
  excretion, 476
  physical properties, 475
Mandelic acid, toxicity, 478
    urogenital infections, 475
Mapharsen and syphilis, 264
  chemotherapeutic index, 264
  constitution, 265
  toxic reactions, 267
  toxicity, 265
Mastitis bovine and acriflavine, 361
    and rivanol, 361
    and sulphanilamide, 361
  human and proseptasine, 411
    and sulphanilamide, 411
  of pigs and sulphonamide compounds, 361
M. & B. 693, (*p*-aminobenzenesulphonamido) pyridine constitution, 328
  in experimental pneumococcal infections, 386
  in gonorrhœa, 405
  in meningococcal septicæmia, 395
  in pneumococcal infections in man, 387
  toxic reactions, 389
  toxicity, 386
Meningitis due to *Bacterium coli* and sulphanilamide, 418
  due to *Hæmophilus influenzæ* and sulphanilamide, 424
  due to *Proteus vulgaris* and sulphanilamide, 424
  gonococcal and sulphanilamide, 402
  meningococcal and sulphonamide compounds, 392
    synergic action of immune serum and sulphonamide compounds, 393
  pneumococcal and sulphanilamide, 388
    and M. & B. 693, 389
  staphylococcal and sulphanilamide, 410
  streptococcal and sulphonamide compounds, 354
Meningococcal meningitis. *See* Meningitis, meningococcal.
  septicæmia and M. & B. 693, 395
  toxins and 4-nitro-4-aminodiphenylsulphoxide, 393
Mercurochrome—220 soluble, 479
  in acute pyelonephritis, 481
  in leprosy, 320
Mercurophen, 479
Mercury compounds and bacterial infections, 479

Mercury in leprosy, 320
  in syphilis, 239, 281
Merthiolate, 479
Metaphen in bacterial infections, 479
  in tularemia, 481
Methæmoglobinæmia and methylene blue, 447
  and plasmoquine, 128
  and sulphonamide compounds, 429, 445
Methylene blue, relation to plasmoquine, 126
    treatment of cyanosis, 447
N-**Methylpsychotrine** as amœbicide, 68
O-**Methylpsychotrine,** constitution, 59
Moranyl identical with germanin, 170
Mycotic infections and alkyl dimethylbenzyl ammonium chlorides, 483
    and arsphenamines, 483
Myocrysin in tuberculosis, 289

Naphthalene derivatives of sulphanilamide, 365
α-**Naphthylamine-4-sulphonic** acid in streptococcal infections, 365
Neoarsphenamine, action on fungi, 483
  composition, 238
  correlation of trypanocidal and spirochæticidal activity, 243
  hypersensitivity, 248
  jaundice, 249
  stability of solutions, 243
  standardization, 241
  toxic reactions, 246
    prevention, 256
Neocryl (Sodium succinanilomethylamide-*p*-arsonate), constitution, 160
  dermatitis, 274
  in cerebrospinal fluid, 162
  in treatment of syphilis, 273
  in treatment of trypanosomiasis, 161
  toxic reactions, 161
  toxicity for animals, 161
Neostam, composition, 89, 91
  in kala azar, 94
Neostibosan, chemotherapeutic index, 93
  composition, 89, 91
  distribution in tissues, 98
  in kala azar, 93
  in oral leishmaniasis of Sudan, 104
Nephritis following sulphanilamide, 448
Nervous symptoms and sulphonamide drugs, 430
Neuritis and uleron, 431
4-Nitro-4-aminodiphenylsulphone in streptococcal infections, 377
4-Nitro-4-aminodiphenylsulphoxide and meningococcal endotoxins, 393
p-**Nitrobenzenesulphonamide** in streptococcal infections, 369
Nitrogen retention and sulphanilamide, 430
Nodal rhythm and sulphanilamide, 448
o-*n*-**Nonylharmol** and amœbicide, 69
Norcephæline hydrochloride as amœbicide, 68
Norconessine as amœbicide, 75
Novostiburea, composition, 90

Olfactory mucosa and prophylaxis of poliomyelitis, 492
Optic atrophy and tryparsamide, 431
    and sulphanilamide, 431
Optochin. *See* Ethyldihydrocupreine.
Oral leishmaniasis in Sudan and neostibosan, 104
Oriental sore and berberine, 101
Orsanine in trypanosomiasis, 159
Osteomyelitis and cod liver oil, 484
Otitis media and sulphanilamide, 354

Paludex in malaria, 118
  in schistosomiasis, 52
Parafilaria multipapillosa and antimonyl tartrates, 57
Parafuchsin and chemotherapeutic interference, 200
Parafuchsin-resistant trypanosomes, 187
Paralysis and gold salts, 292
Pasteurella infections and sulphonamide compounds, 421
Pemphigus and sulphanilamide, 356
Pentachloroethane in ankylostomiasis, 35
  toxicity, 35
Peritonitis and sulphanilamide, 352
Phagocytes and sulphonamide compounds, 458
Phenanthridinium compounds in trypanosomiasis, 181
Phenylalanine sulphonamide in streptococcal infections, 370
Phenylhydrazine-*p*-sulphonic acid in streptococcal infections, 365
Photosensitivity and sulphanilamide, 434
Picric acid and alum in prophylaxis of poliomyelitis, 492
Plague and prontosil, 422

Plasmodium knowlesi and sulphonamide compounds, 152
Plasmoquine, action on parasites, 130
  and blackwater fever, 153
  and infectivity of parasites, 150
  and methæmoglobinæmia, 128
  and prophylaxis of malaria, 129
  chemotherapeutic index, 127
  constitution, 127
  estimation, 129
  homologues in bird malaria, 133
  in pregnancy, 130
  relation to methylene blue, 126
   to quinoline, 127
  toxic reactions, 128
Plasmoquine-atebrin combination in malaria, 149
Plasmoquine-quinine combination in malaria, 149
Platinum in syphilis, 284
Pneumococcal infections and benzylidene Schiff's base of 4 : 4′-diaminodiphenylsulphone, 384
   and 4: 4′-diaminodiphenylsulphone, 385
   and M. & B. 693, 386
   and prontosil, 284
   and rodilone, 385
   and sulphanilamide, 384
   and uleron, 385
  meningitis and sulphanilamide, 388
   and M. & B. 693, 389
  peritonitis and M. & B. 693, 389
  septicæmia and M. & B. 693, 389
Pneumococci and quinine derivatives, 470
  capsule formation and M. & B. 693, 388, 456
  growth *in vitro* with M. & B. 693, 389
   with sulphanilamide, 384
  types and sulphanilamide, 388
Pneumonia and *apo*-quinine derivatives, 470
  and ethyldihydrocupreine (optochin), 470
  and M. & B. 693, 388
  and sulphanilamide, 387
  streptococcal and sulphonamide drugs, 355
Poliomyelitis and sulphonamide compounds, 486
  and potassium chlorate, 492
  and prophylaxis by nasal spray, 492
Polyneuritis and bismuth, 282
Porphyrins and sulphanilamide, 433
Potassium antimonyl tartrate in schistosomiasis, 44
   toxicity, 45
  chlorate in poliomyelitis, 492
Prontosil, active constituent, 323
  and complications of influenza, 356
  and empyema, 356
  and erysipelas, 350
  and Ludwig's angina, 354
  and otitis media, 355
  and plague, 422
  and pneumococcal infections of laboratory animals, 383
  and pneumonia, 356
  and puerperal fever, 346
   prophylaxis, 348
  and rheumatoid arthritis, 352
  and septicæmia, 350
  and streptococcal pneumonia, 355
   peritonitis, 352
   pyelitis, 349
  and tonsillitis, 353
  and typhus fever, 492
  base, 324
  constitution, 324
  dosage, 426
  estimation, 341
  in cerebrospinal fluid, 332
  methods of administration, 426
  pathological lesions, 340
  pharmacology, 329
  physical properties, 324
  streptococcal infections of laboratory animals, 322, 345
   meningitis, 355
Prontosil soluble and arthritis, 352
   and infected wounds, 352
   and lymphadenitis of guinea-pigs, 361
   and puerperal fever, 347
   and streptococcal infections in mice, 363
   constitution, 325
   excretion, 331
   in cerebrospinal fluid, 332
   pharmacology, 329
   synonyms, 324
  synonyms, 323
  synthesis, 322
  toxic reactions, 428
  toxicity, 339
Proparsamide in amœbiasis, 83
n-**Propyl** chloride in ascariasis, 38
   physical properties, 38
  iodide in ascariasis, 38

Propylene chloride in ascariasis, 40
o-n-**Propylharmol** as amœbicide, 69
Proseptasine and erysipelas, 350
  and gonorrhœa, 402
  and puerperal fever, prophylaxis, 348
  and scarlet fever, 354
  and streptococcal infections in mice, 363
  constitution, 327
  toxicity, 339
Proteus vulgaris and mandelic acid, 477
    in genito-urinary infections and sulphanilamide, 417
    meningitis and sulphanilamide, 424
Pseudomonas and mandelic acid, 477
Psittacosis and acriflavine, 492
Psychotrine, constitution, 59
Puerperal fever and prontosil, 346
    prontosil soluble, 347
    and proseptasine, 348
    and prophylaxis, 348
    and rubiazol, 348
    and sulphanilamide, 347
  infection of dog and sulphanilamide, 361
Pyelitis due to *Bacterium coli* and sulphonamide compounds, 415
  due to *Bacterium typhosum* and sulphonamide compounds, 419
  due to Staphylococci and sulphonamide compounds, 411
  due to Streptococci and sulphonamide compounds, 349
Pyoktanin and chemotherapeutic interference, 200
Pyorrhœa and sulphonamide compounds, 424
Pyridine arsenicals in syphilis, 274
    in trypanosomiasis, 167
  compounds in streptococcal infections, 468
Pyridium as urinary antiseptic, 469
Pyrocatechol compounds in schistosomiasis, 47
Pyrolle dyes in trypanosomiasis, 181

Quinacrine identical with atebrin, 137
Quinimax in human malaria, 121
Quinine, action on malaria parasites, 123
  and blackwater fever, 152
  derivatives and pneumococci, 470
  efficiency in bird malaria, 110
  in human malaria, 120
  in malaria prophylaxis, 122
Quinine-atebrin in human malaria, 149
Quinine-plasmoquine in human malaria, 149
Quinoline derivatives in amœbiasis, 70
    in trypanosomiasis, 175
  group in cinchona alkaloids, 108
Quinquevalent antimony compounds and dermal leishmaniasis, 101
    and muco-cutaneous leishmaniasis, 103
    and oral leishmaniasis, 103
    compared with tervalent preparations, 96
    in kala-azar, 93
  arsenicals, action in syphilis, 297
Quinsy and sulphonamide drugs, 354
Quinuclidine group in cinchona alkaloids, 108

Reactions to arsphenamines, prevention and treatment, 256
  to sulphonamide drugs, prevention and treatment, 449
Redox potentials and chemotherapeutic interference, 201
Reduced tryparsamide and drug resistance, 187
Reticulo-endothelial system and anti-malarial drugs, 116
    and chemotherapy of trypanosomiasis, 231
Rheumatoid arthritis and sulphanilamide, 353
Rhodoquine in bird malaria, 133
Rift Valley fever and sulphonamide drugs, 487
Rivanol in amœbiasis, 85
  in bovine mastitis, 361
Rodilone in gonorrhœa, 403
  in pneumococcal infections, 385
  in streptococcal infections, 372
Rubiazol, constitution, 325
  in glanders, 424
  in prophylaxis of puerperal fever, 348
  nomenclature, 325

Sanocrysin in tuberculosis, 288
  toxicity, 289
Santonin in ascariasis, 10
  structure, 10
  syntonins a and b, 11
Sapucainha oil, physical properties, 300, 301
Scarlet fever and prospetasine, 354
    and sulphanilamide, 354
      prophylaxis, 354

Schiff's base of 4 : 4′-diaminodiphenylsulphone in streptococcal infections, 376
Schistosomiasis and antimonyl tartrates, 44
  and antimosan, 47
  and copper salts, 52
  and criteria of cure, 53
  and dicuprène, 52
  and Dn. 7 (stabilase), 51
  and Dn. 18 (tristibine), 51
  and fouadin, 47
  and lithium antimonyl tartrate, 46
  and paludex, 52
  and pyrocatechol compounds, 47
  and quinquevalent antimony compounds, 51
  and tartar emetic, 44
Schistosomum japonicum and fouadin, 48
Sdt. 386 B in *Bartonella* infections, 483
  in oriental sore, 102
  in schistosomiasis, 56
  in streptococcal infections, 483
Septicæmia, staphylococcal, and sulphanilamide, 410
  streptococcal, and sulphonamide drugs, 349
Serum-resistant trypanosomes, 188
Silver arsphenamine, 241
  neoarsphenamine, 241
Sinusitis and sulphanilamide, 354
Sniffles of rats and sulphanilamide, 424
Sodium-4-acetylamino-2-hydroxyphenylarsonate (orsanine) in trypanosomiasis, 159
  toxicity, 160
Sodium antimonyl tartrate in schistosomiasis, 45
Sodium arsphenamine, 241
Sodium-dichaulmoogryl-β-glycerophosphate (chaulphosphate) in leprosy, 304, 308, 310
Sodium gynocardate, toxicity, 305
Sodium N-phenyl-β-aminoproprionamide-*p*-arsonate (proparsamide) in amœbiasis, 83
Sodium salt of 3-amino-4-β-hydroxyethoxyphenylarsonic acid in syphilis, 273
Sodium salts of the fatty acids of *Hydnocarpus Wightiana* in leprosy, 308, 310
Sodium succinanilomethylamide-*p*-arsonate (neocryl) in trypanosomiasis, 160
    in syphilis, 273
Sodium sulphanilyl sulphanilate in canine distemper, 489
Sodium thiosulphate and tuberculosis, 294
Solganal and tuberculosis, 289
Solganal B and leprosy, 320
  and tuberculosis, 289
  oleosum and leprosy, 320
    and tuberculosis, 289
Solusalvarsan and syphilis, 263
  toxicity, 263
Soluseptasine, constitution, 327
  in experimental streptococcal infections, 364
  in gonorrhœa, 402
  toxicity, 339
Spirillum minus and anil- and styrylquinolines, 284
Staphylococcal infections and 4 : 4′-diaminodiphenylsulphone, 410
    and disulphanilamide, 409
    and mandelic acid, 477
    and prontosil, 410
    and sulphanilamide, 409
    and uleron, 368, 410
  mastitis and sulphonamide drugs, 411
  meningitis and sulphanilamide, 410
  septicæmia and sulphanilamide, 410
    and uleron, 412
Stereochemical relations of cinchona alkaloids, 109
Stibacetin or stibenyl in kala-azar, 89
Stibamine in kala-azar, 89
Stibosan in kala-azar, 89
Stovarsol. *See* Acetarsol.
Streptococcal infections in domestic animals and sulphonamide compounds, 360
    in mice and α-acetylamino-naphthalene-4-sulphonamide, 365
    in mice and acridine derivatives, 465
      and *p*-aminobenzene-sulphonanilide, 365
      and *m*-aminobenzene-sulphonic acid, 365
      and aurodetoxin, 464

Streptococcal infections in mice
- and 4 : 4′-diacetylaminodiphenylsulphone (rodilone) 372
- and 4 : 4′-diaminodiphenylsulphide, 378
- and 4 : 4′-diaminodiphenylsulphone, 375
- and 4 : 4′-diaminodiphenylsulphoxide, 375
- and 4 : 4′-dinitrodiphenylsulphide, 378
- and 4 : 4′-dinitrodiphenylsulphone, 375
- and diphenylsulphides, 371
- and diphenylsulphones, 371
- and disulphanilamide, 367
- and glucose derivative of 4 : 4′-diaminodiphenylsulphone, 387
- and M. & B. 693, 386
- and *α*-naphthylamine-4-sulphonic acid, 365
- and 4-nitro-4-aminodiphenylsulphone, 377
- and *p*-nitrobenzenesulphonamide, 369
- and phenylalaninesulphonamide, 370
- and phenylhydrazine-*p*-sulphonic acid, 365
- and prontosil, 322, 345
- and prontosil soluble, 363
- and proseptasine, 363
- and pyridine compounds, 468
- and soluseptasine, 364
- and *p*-sulphamidophenylpyridine-*β*-carboxyamide-*α*-carboxylic acid, 369, 468
- and sulphanil-*p*-aminoanilide,369
- and sulphanil-*p*-nitroanilide, 369
- and sulphanilamide, 345
- and sulphanilic acid, 365
- and sulphinic acid, 482
- and sulphonic acid compounds of *p*-aminobenzene sulphonamide, 370
- and *p*-toluene sulphonamide, 365
- and *p*-toluenesulphonethylamide, 365
- and trisulphanilamide, 369

Streptococci, anaerobic and sulphonamide compounds, 346
- groups and reaction to sulphonamide compounds, 345

Streptococci, non-hæmolytic and mandelic acid, 477

Strontium in syphilis, 284

Styryl benzthiazole derivatives and trypanosomiasis, 175
- derivatives of 4-aminoquinaldine, 176
- quinoline derivatives and drug-resistance, 187
  - and trypanosomiasis, 175

Sugar metabolism and trypanocidal drugs, 183

Sulphæmoglobinemia and sulphonamide compounds, 447

Sulphamidoazobenzeneazohydrocupreines, bacterial action *in vitro*, 323

Sulphanil-*p*-aminoanilide and streptococcal infections, 369

Sulphanil-*p*-nitroanilide and streptococcal infections, 369

Sulphanilamide, active constituent of prontosil, 323
- activity in anaerobic streptococcal infections, 346
  - in hæmolytic streptococcal infections, 345
  - in non-hæmolytic streptococcal infections, 345
- and acetylation by liver, 334
- and acetylsulphanilamide relationship, 333
- and acidosis, 420
- and actinomycosis, 420
- and adenitis, 361
- and agranulocytosis, 437
- and alkali reserve, 335
- and anæmia, 441
- and appendicitis, 424
- and arthritis, 352
- and *Bacterium coli*, 415
- and *Bacterium paratyphosum* B, 419
- and *Bacterium typhosum*, 419
- and balanitis, 424
- and blood pigments, 444
- and boils, 411
- and bone marrow, 384
- and bovine mastitis, 361
- and canine distemper, 361, 489
- and carbuncles, 411
- and chancroid, 422
- and *Clostridium welchii*, 420
- and common cold, 487
- and diphtheroids, 423
- and Ducrey's bacillus, 422
- and feline distemper, 361, 487
- and filarial lymphangitis, 57

Sulphanilamide and furuncles, 411
  and genito-urinary infections due to *Bacterium coli*, 415
      due to *Proteus vulgaris*, 417
      due to *Streptococcus fæcalis*, 417
  and gonococcal infections, 398
    meningitis, 402
    ophthalmia neonatorum, 402
    rheumatism, 402
    vulvo-vaginitis, 401
  and gonorrhœa, 398
  and growth of *Pneumococci in vitro*, 384
  and hæmatopoietic system, 463
  and *Hæmophilus influenzæ*, 423
  and *Hæmophilus pertussis*, 423
  and hypersensitivity, 247
  and jaundice, 448
  and liver abscess due to *Bacterium coli*, 418
  and Ludwig's angina, 353
  and lymphocytic choriomeningitis, 487
  and lymphogranuloma inguinale, 490
  and malaria, 152
  and mandelic acid compared, 417
  and mastitis in man, 411
    in the cow, 361
    in the pig, 361
  and meningitis due to *Bacterium coli*, 418
      to *Gonococcus*, 402
      to *Hæmophilus influenzæ*, 424
      to *Meningococcus*, 392
      to *Pneumococcus*, 388
      to *Proteus vulgaris*, 424
      to *Staphylococcus*, 410
      to *Streptococcus hæmolyticus*, 354
      to *Str. viridans*, 355
  and nephritis, 448
  and nitrogen retention, 430
  and nodal rhythm, 448
  and optic atrophy, 431
  and otitis media, 354
  and pemphigus, 356
  and peritonitis, 352
  and photosensitivity, 434
  and pneumococcal infections, 384
    meningitis, 388
  and pneumococci types, 388
  and pneumonia, 387
  and porphyrins, 433
  and *Proteus vulgaris* infections, 417, 424
  and puerperal fever, 347
    infection of dog, 361

Sulphanilamide and pyelitis due to *Bacterium coli*, 416
    to streptococci, 349
  and pyorrhœa, 424
  and rabbit fibroma virus, 487
  and scarlet fever, 354
  and septicæmia, staphylococcal, 411
  and sinusitis, 354
  and sniffles of rats, 424
  and streptococcal infections, experimental, 345
      in domestic animals, 360
      in man, 346
  and thrombocytopenia, 443
  and tonsillitis, 353
  and trachoma, 491
  and tuberculosis, 295
  and typhoid fever, 419
    pyelitis, 419
  and virus myxomatosum of rabbits, 487
  and yellow fever virus, 487
  constitution, 325
  distribution in tissues, 331
  dosage, 427
  estimation, 341
  excretion, 333
  in solution, 327
  methods of administration, 427
  modification of structure and therapeutic action, 364
  naphthalene derivatives, 365
  pharmacology, 329
  physical properties, 327
  sulphæmoglobinæmia, 447
  toxic reactions in cow, 362
      in horse, 362
      in man, 428
      prevention, 449
  toxicity for laboratory animals, 337
Sulphanilic acid and streptococcal infections, 365
Sulpharsphenamine, biological standardization, 242
  relation to trisodarsen, 269
Sulphinic acids and streptococcal infections, 482
Sulphonamide compounds, action on bacteria, 454
      on gonococci *in vitro*, 398
    and bacterial capsules, 456
      endocarditis, 356
    and bactericidal action, 454
    and bacteriostasis, 454

Sulphonamide compounds and *Bacterium aerogenes*, 423
  and Brucella infections in animals, 415
    in man, 413
  and cellulitis, 351
  and cyanosis, 444
  and drug fever, 432
  and empyema, 355
  and erysipelas, 350
  and gas gangrene, 420
  and gonococcal endotoxins, 406
    infections, 406
  and herpes virus, 486
  and impetigo contagiosa, 356
  and influenza virus, 487
  and jaundice, 448
  and lymphocytic choriomeningitis, 487
  and lymphogranuloma inguinale, 490
  and lupus erythematosus, 356
  and meningococcal meningitis, 392
  meningococcal meningitis and immune serum, 393
  and methæmoglobinæmia, 447
  and nervous symptoms, 430
  and Pasteurella infections, 421
  and phagocytosis, 458
  and *Plasmodium knowlesi*, 152
  and spermatogenesis, 448
  and streptococcal pneumonia, 355
    septicæmia, 349
  and poliomyelitis, 486
  and pyelitis due to *Bacterium coli*, 415
    Bacterium typhosum, 419
    to *Staphylococcus*, 411
    to *Streptococcus*, 349
  and pyorrhœa, 424
  and tonsillitis, 353
  and toxic reactions, 428
    prevention, 449
  and toxins, neutralization, 457
  and variation in bacterial susceptibility, 451
  and yellow fever, 487
  correlation of chemical structure and therapeutic action, 451
  history, 447
Sulphonic acid compounds of *p*-aminobenzenesulphonamide, 370
Sulphydryl group and gold salts, 294
  and trypanocidal action, 223
Surfen C and trypanosomiasis of animals, 177
  resistant trypanosomes, 188
Synergic action of sulphonamide compounds and immune serum, 389, 393, 410
  of trypanocidal drugs, 229
Synthalin and trypanosomiasis, 183
Synthalin B and trypanosomiasis, 183
Syphilis, action of ter- and quinquevalent arsenicals, 277
  and aldarsone, 272
  and arseno-pyridines, 274
  and bismuth, 279
  and continuous treatment, 239
  and drug-resistance, 283
  and intermittent treatment, 239
  and mapharsen, 264
  and neocryl, 273
  and rhodium, 284
  and sodium salt of 3-amino-4-β-hydroxyethoxyphenylarsonic acid, 273
  and solusalvarsan, 263
  and strontium, 284
  and thioarsene, 271
  and trisodarsen, 269
  jaundice and arsphenamines, 252
  prophylaxis, 285

Tartar emetic and kala-azar, 87
  and *Parafilaria multipapillosa*, 57
  and schistosomiasis, 43
  and toxic reactions, 45
  and trypanosomiasis, 167
Tervalent and quinquevalent antimony in kala-azar, 96
    arsenicals in syphilis, 263, 277
  arsenicals, chemotherapeutic indices in trypanosomiasis, 236
Tetrachloroethane, toxicity, 35
Tetrachloroethylene and ankylostomiasis, 31
  and ascariasis, 33, 37
  and hook worms of silver foxes, 33
  and physical properties, 31
  and toxicity, 31
Thioarsene in syphilis, 271
Thioarsinites and trypanocidal action, 214
Thrombocytopenia and sulphanilamide, 443
Thymol in ankylostomiasis, 25
p-**Toluenesulphonamide** in streptococcal infections, 365, 366

p-**Toluenesuplhonethylamide,** 365
Tonsillitis and prontosil, 353
  and sulphanilamide, 353
Totaquina composition, 124
  in malaria, 125
  relation to cinchona febrifuge, 124
Toxic reactions and arsphenamines, 246
      prevention, 256
    and sulphonamide compounds, 428
      prevention, 449
Toxins and neutralization by sulphonamide compounds, 457
Trachoma and antimony salts, 491
  and quinine, 492
  and sulphanilamide, 491
Trichloroethane as anthelmintic, 40
Trichomonas and carbarsone, 83
Triphal in tuberculosis, 289
Trisodarsen, relation to sulpharsphenamine, 269
  in syphilis, 269
  toxic reactions, 269
Tristibine in schistosomiasis, 51
Trisulphanilamide in streptococcal infections, 369
Trypan blue in leprosy, 314
    toxicity, 315
Trypanocidal action and cysteine, 215
    and glutathione, 213
    and reticulo-endothelial system, 231
    and synergism, 229
    and thioarsinites, 214
    in vitro and *in vivo*, 210
    of germanin, 216
    of quinquevalent arsonic acids, 211
    of tervalent arsenicals, 211
  drugs, absorption by trypanosomes, 222
    rate of absorption by tissues, 218
      of excretion by tissues, 218
Trypanosomes, action of drugs on, 210
    and chemotherapeutic interference, 200
  and drug-resistance, 187
      mechanism, 193
      stability, 191
  and oxygen uptake, 225
  and reversal of electric charge, 231
  and specific relation to arsenicals,227
  and spontaneous change in reaction to drugs, 230
  and sugar metabolism, 183
  in tsetse flies, action of drugs, 234
        stability of drug-resistance, 191
Trypanosomiasis, treatment by acridine derivatives, 180
    by anil-and styryl-quinoline derivatives, 175
    by antimony preparations, 167
    by antimosan, 168
    by aromatic arsonic acids with amide groups, 166
    by arsenic brown, 167
      yellow, 167
    by arsenical derivatives of pyridine, 167
    by arseno-detoxins, 166
    by arylthioarsinites, 163
    by diarsonic acids, 163
    by Dn. 9, 168
    by Dn. 12, 168
    by Dn. 18, 168
    by germanin, 171
    by guanidine derivatives, 183
    by K. 324, 165
    by K. 325, 165
    by lithium antimony thiomalate, 168
    by neocryl, 160
    by orsanine, 159
    by phenanthridinium compounds, 181
    by quinquevalent arsenicals, 155
    by surfen C, 177
    by synthalin, 183
    by tartar emetic, 167
    by tryparsamide, 157
    by N-Undecamine 1 : 11-diamidine 2HCl, 185
  prophylaxis by germanin, 204
    by benzolyaminostyrylquinoline compounds, 207
Tryparsamide and optic atrophy, 156
  composition, 155
  in treatment of trypanosomiasis, 157
  test for toxicity, 156
Tryparsamide-resistant trypanosomes, 190
Tsetse flies, action of drugs on trypanosomes in, 234
    effect of passage of drug-resistant trypanosomes, 191
Tuberculosis, treatment by allochrysine, 289
    by cadmium, 294
    by calcium salts, 286
    by gold salts, 288
    by heavy metals, 294
    by lopion, 289

Tuberculosis, treatment by myocrysin, 289
    by sanocrysin, 288
    by sodium thiosulphate, 294
    by solganal, 289
    by solganal B, 289
    by sulphanilamide, 295
Tularemia and metaphen, 481
Typhoid fever and sulphanilamide, 419
  pyelitis and sulphanilamide, 419
Typhus and prontosil, 492

Uleron and neuritis, 431
  constitution, 327, 368
  in gonorrhœa, 403
  in meningococcal meningitis, 393
  in staphylococcal infections, 410
  in streptococcal infections, 368
  in toxicity, 344
  in urogenital infections, 418
N-Undecamine 1 : 11 diamidine 2HCl
      and drug-resistant trypanosomes, 188
    in treatment of malaria, 186
      of trypanosomiasis, 188
    toxicity, 185
Undulant fever. *See Brucella* infections.
Urea and wound healing, 484
Urea-stibamine, constitution, 89
  in kala-azar, 90
Urea-stibol, constitution, 89

Vioform (iodochlorohydroxyquinoline) in amœbiasis, 72
Virus myxomatosum of rabbits and sulphanilamide, 487
Viruses, chemoprophylaxis, 492
  chemotherapy, 486

Wound healing and allantoin, 484
    and cod liver oil, 484
    and urea, 484

Xanthoxylum schinifolia oil in ascariasis, 9

Yatren (iodohydroxyquinolinesulphonic acid) in amœbiasis, 70
Yeasts and chemotherapeutic interference, 201
Yellow fever and sulphonamide compounds, 487

Zinc sulphate in prophylaxis of poliomyelitis, 493

PRINTED IN GREAT BRITAIN BY THE WHITEFRIARS PRESS LTD.
LONDON AND TONBRIDGE

No. 2.

J. & A. CHURCHILL
LIMITED

Selected Books for Students and Practitioners of Medicine

LONDON
104 Gloucester Place,
Portman Square, W. 1.

INDEX

PAGE
2 Anatomy. Physiology. Biochemistry.
3 Materia Medica. Pharmacy.
4 Hygiene. Bacteriology.
5 Pathology. Psychology. Dictionaries. Town Planning.
6 Medicine.
7 Medicine. Massage. Gymnastics.
8 Surgery. Radiology.
9 Surgery. Anæsthetics.
10 Dermatology. Neurology. Urinary Disorders. Tropical Diseases.
11 Medical Jurisprudence and Toxicology. Ophthalmology.
12 Midwifery. Gynæcology.
13 Pædiatrics. Otology. Dentistry.
14 Chemistry.
15 Chemistry.
16 Physics. Microscopy. Biology.

Anatomy & Physiology Biochemistry

Elementary Anatomy and Physiology. By James Whillis, M.D., M.S., F.R.C.S., University Reader in Anatomy, Guy's Hospital Medical School. 87 Original Illustrations. 12*s*. 6*d*. net.

Surgical Anatomy. By Grant Massie, F.R.C.S. Third Edition. 153 Illustrations, some in colour. 18*s*. net.

The Anatomy of the Human Skeleton. By J. Ernest Frazer, F.R.C.S. Fourth Edition. 219 Illustrations. 30*s*. net.

Synopsis of Regional Anatomy. By T. B. Johnston, M.D., Professor of Anatomy, Univ. of London. Fourth Edition. 17 Illus. 12*s*. 6*d*. net.

Text-Book of Anatomy and Physiology for Nurses. By E. R. Bundy, M.D. Sixth Edition. 266 Illustrations. 12*s*. net.

Experimental Physiology for Medical Students. By D. T. Harris, M.D., D.Sc., F.Inst.P. Second Edition. 1 Colour Plate and 236 Text-figures. 12*s*. 6*d*. net.

Starling's Principles of Human Physiology. Seventh Edition. Edited by C. Lovatt Evans, D.Sc., F.R.C.P., F.R.S., Jodrell Professor of Physiology, University College, London. 554 Illus. 24*s*. net.

An Introduction to Biophysics. By D. Burns, D.Sc., Professor of Physiology, Univ. of Durham. 2nd Ed. 116 Illus. 25*s*. net.

Human Physiology. By F. R. Winton, M.D., and L. E. Bayliss, Ph.D. Second Edition. 221 Illustrations. 15*s*. net.

Recent Advances in Physiology (Lovatt Evans). Sixth Edition revised by W. H. Newton, M.D., M.Sc. 109 Illustrations. 15*s*. net.

The Mechanism of the Human Voice. By Robert Curry, M.A., Ph.D. 20 Illustrations. 10*s*. 6*d*.

Recent Advances in Biochemistry. By J. Pryde, D.Sc. Third Edition. 42 Illustrations. 12*s*. 6*d*. net.

A Text-Book of Biochemistry for Students of Medicine and Science. By A. T. Cameron, M.A., D.Sc., F.I.C., F.R.S.C. Fifth Edition. 3 Plates and 25 Text-figures. 15*s*. net.

A Course in Practical Biochemistry. By Prof. A. T. Cameron and Frank White, A.R.T.C., Ph.D. Third Edition. 4 Plates and 23 Figures. 8*s*. 6*d*. net.

Biochemistry of Medicine. By A. T. Cameron and C. R. Gilmour, M.D., C.M. Second Edition. 31 Illustrations. 21*s*. net.

Recent Advances in Sex and Reproductive Physiology. By J. M. Robson, M.D., B.Sc. 47 Illustrations. 12*s*. 6*d*. net.

The Physiology of Human Perspiration. By Yas Kuno. 38 Illustrations. 12*s*. 6*d*. net.

Practical Physiological Chemistry. By P. B. Hawk, M.S., Ph.D., and Olaf Bergeim, M.S., Ph.D. Eleventh Edition. 288 Illustrations. 35*s*. net.

Biochemistry for Medical Students. By W. V. Thorpe, M.A., Ph.D., Reader in Chemical Physiology, University of Birmingham. 2nd Edition. 4 Plates and 33 Text-Figures. 12*s*. 6*d*. net.

J. & A. CHURCHILL Ltd.

Materia Medica & Pharmacy

Recent Advances in Chemotherapy. By G. M. Findlay, *C.B.E.*, M.D., D.Sc. Second Edition. 21

Essentials of Materia Medica, Pharmacology and Therapeutics. By R. H. Micks, M.D., F.R.C.P.I. Second Edition. 12*s.* 6*d.* net.

Recent Advances in Materia Medica (Sera, Vaccines, Hormones and Vitamins). By J. H. Burn, M.D. 25 Illustrations. 12*s.* 6*d.* net.

Applied Pharmacology. By A. J. Clark, *M.C.*, M.D., F.R.C.P., Professor of Materia Medica, University of Edinburgh. Sixth Edition. With 83 Illustrations. 18*s.* net.

Cushny's Text-Book of Pharmacology and Therapeutics. Eleventh Edition. By C. W. Edmunds, M.D., and J. A. Gunn, M.D., F.R.C.P., D.Sc. 70 Illustrations. 25*s.* net.

Hale-White's Materia Medica, Pharmacy, Pharmacology and Therapeutics. Twenty-fourth Edition. Revised by A. H. Douthwaite, M.D., F.R.C.P. 12*s.* 6*d.* net.

Cow's Synopsis of Pharmacology. Second Edition. Revised by G. Norman Myers, M.D. With 15 Illustrations. 7*s.* 6*d.* net.

A Text-Book of Pharmacognosy. By Henry G. Greenish, F.I.C., F.L.S. Sixth Edition. 297 Illustrations. 25*s.* net.
An Anatomical Atlas of Vegetable Powders. 138 Illustrations. 12*s.* 6*d.* net.

Practical Pharmacognosy. By T. E. Wallis, B.Sc., F.I.C., Ph.C. Third Edition. 72 Illustrations. 12*s.* 6*d.* net.

Principles of Pharmacy. By H. B. Mackie, B.Pharm., Ph.C. 67 Illustrations. 10*s.* 6*d.* net.

The Science and Practice of Pharmacy. By R. R. Bennett, B.Sc., F.I.C., and T. Tusting Cocking, F.I.C. Vol. I. Pharma-

10*s.* 6*d.* net. With an Appendix comprising Notes on the 1936 Addendum to the 1932 B.P.

First Lines in Dispensing. By H. B. Stevens and C. E. L. Lucas, A.I.C., F.C.S. Third Edition. 95 Illustrations. 7*s.* 6*d.* net.

Favourite Prescriptions, Including Dosage Tables, Hints for Treatment of Poisoning and Diet Tables. By Espine Ward, M.D. (Belfast). Fourth Edition. Interleaved. 7*s.* 6*d.* net.

By E. W. Lucas, C.B.E., F.I.C., and H. B. Stevens, O.B.E., F.I.C.

Book of Pharmacopœias and Unofficial Formularies. 7*s.* 6*d.* net.

The Book of Receipts. Twelfth Edition. 10*s.* 6*d.* net.

The Book of Prescriptions, with an Index of Diseases and Remedies. Eleventh Edition. 10*s.* 6*d.* net.

The Pharmacological Action of the Harrogate Drinking Waters. By W. Bain, M.D., F.R.C.P., F.R.C.S. 26 Illustrations. 5*s.* net.

Hygiene Bacteriology

Recent Advances in Industrial Hygiene and Medicine. By T. M. Ling, B.M., M.R.C.P. 29 Illustrations. 12*s*. 6*d*. net.

Synopsis of Hygiene. By Sir W. Wilson Jameson, M.D., F.R.C.P., D.P.H., Professor of Public Health, London University, and G. S. Parkinson, *D.S.O.*, M.R.C.S., L.R.C.P., D.P.H., Assistant Director, Public Health Division, London School of Hygiene and Tropical Medicine. Sixth Edition. 16 Illustrations. 21*s*. net.

The Health of the Industrial Worker. By E. L. Collis, M.D., and Major Greenwood, M.R.C.P., M.R.C.S. 30*s*. net.

The Principles of Preventive Medicine. By R. Tanner Hewlett, M.D., F.R.C.P., D.P.H. With 12 Charts and 5 Diagrams. 18*s*. net.

A Simple Method of Water Analysis. By J. C. Thresh, M.D., D.P.H., and J. F. Beale, M.R.C.S., D.P.H. Tenth Edition. 3*s*. net.

Elementary Hygiene for Nurses. By H. C. Rutherford Darling, M.D., F.R.C.S. Seventh Edition. 58 Illustrations. 6*s*. net.

Preservatives in Food and Food Examination. By J. C. Thresh and A. E. Porter, M.D. 8 Plates. 16*s*. net.

Text-book of Meat Hygiene. By R. Edelmann, Ph.D. Translated by J. R. Mohler, A.M., V.M.D., and A. Eichhorn, D.V.S. Sixth Edition. With 162 Illustrations and 5 Plates. 28*s*. net.

A Manual of Bacteriology: Medical and Applied. By Prof. R. T. Hewlett, M.D., F.R.C.P., D.P.H., and Prof. J. McIntosh, M.D., B.Ch. Ninth Edition. 43 Plates and 66 Text-figures. 18*s*. net.

The Chemical Analysis of Foods. By H. E. Cox, D.Sc., Ph.D., F.I.C. Second Edition. 41 Illustrations. 21*s*. net.

Medical Bacteriology, including Elementary Helminthology. By L. E. H. Whitby, *C.V.O.*, M.D., F.R.C.P. Third Edition. 79 Illustrations. 11*s*. 6*d*. net.

Recent Advances in Bacteriology. By J. H. Dible, M.B., Ch.B., F.R.C.P. Second Edition. 29 Illus. 15*s*. net.

Blood Cultures and Their Significance. By Hildred M. Butler, B.Sc., Bacteriologist, Baker Institute of Medical Research, Melbourne. 3 Plates. 15*s*. net.

Recent Advances in Preventive Medicine. By J. F. C. Haslam, *M.C.*, M.D., M.R.C.P., D.P.H. 30 Illustrations 12*s*. 6*d*. net.

Dairy Bacteriology. By Dr. Orla-Jensen. Translated by P. S. Arup, B.Sc., F.I.C. Second Edition. 67 Illustrations. 18*s*. net.

Clinical Bacteriology. By F. A. Knott, M.D., M.R.C.P., D.P.H., Director, Bacteriological Dept., and Lecturer in Bacteriology, Guy's Hospital. 60 Illustrations. 12*s*. 6*d*. net.

Pathology Psychology
Dictionaries Town Planning

Pathology: An Introduction to Medicine and Surgery. By J. H. Dible, M.B., F.R.C.P., and T. B. Davie, M.D., M R.C.P. 374 Illustrations. 36*s*. net.

Recent Advances in Pathology. By G. Hadfield, M.D., F.R.C.P., and Lawrence P. Garrod, M.D., F.R.C.P. Third Edition. 65 Illustrations. 15*s*. net.

Clinical Pathology and the Technique of Collecting Specimens. By William Smith, M.D. 47 Illustrations. 5*s*. net.

Clinical Pathology. By P. N. Panton, M.B., and J. R. Marrack, M.D. Fourth Edition. 12 Plates (10 Coloured) and 50 Text-figures. 16*s*. net.

The Origin of Cancer. By J. P. Lockhart-Mummery, M.B., F.R.C.S. 29 Illustrations. 10*s*. 6*d*. net.

The Spread of Tumours in the Human Body. By R. A. Willis, M.D., B.S., D.Sc. 103 Illustrations. 25*s*. net.

A Handbook of Clinical Chemical Pathology. By F. S. Fowweather, M.D., M.Sc. 18 Illustrations. 8*s*. 6*d*. net.

Modern Psychology in Practice. By W. Lindesay Neustatter, M.B., B.S. With Foreword by R. D. Gillespie, M.D., F.R.C.P., D.P.M. 10*s*. 6*d*.

Recent Advances in Psychiatry. By H. Devine, *O.B.E.*, M.D. Second Edition. 12*s*. 6*d*. net.

Psychological Medicine. By Sir M. Craig, M.D., and T. Beaton, O.B.E., M.D. Fourth Edition. 25 Plates. 21*s*. net.

Recent Advances in Study of the Psychoneuroses. By Millais Culpin, M.D., F.R.C.S. 12*s*. 6*d*. net.

Clinical Lectures on Psychological Medicine. By Henry Yellowlees, *O.B.E.*, M.D., F.R.C.P. 12*s*. 6*d*. net.

The Journal of Mental Science. Published Bi-monthly, by Authority of the Royal Medico-Psychological Association. 6*s*. net.

Lang's German-English Dictionary of Terms used in Medicine and the Allied Sciences. Fourth Edition, edited and revised by M. K. Meyers, M.D. 45*s*. net.

Medical Vocabulary and Phrases in English, German, French, Italian and Spanish. By Joseph S. F. Marie. 12*s*. 6*d*.

A Dictionary of Dental Science and Art. By W. Dunning, D.D.S., F.A.C.D., and S. Ellsworth Davenport, Jr., D.M.D., F.A.C.D. 79 Illustrations. 28*s*. net.

Recent Advances in Town Planning. By Thomas Adams, F.S.I., P.P.T.P.I., and Collaborators. With 2 Coloured Maps and 87 Illustrations. 25*s*. net.

Outline of Town and City Planning. By Thomas Adams, D.Eng., F.R.I.B.A. 126 Illustrations. 18*s*. net.

Medicine

Medicine: Essentials for Practitioners and Students. By G. E. BEAUMONT, Physician, Middlesex Hospital. Third Edition 74 Illustrations. 21*s.* net.

By the same Author.

Diets for Diabetes, Arranged in Menu Form. 2*s.* net.

Medical Emergencies. By CHARLES NEWMAN, M.D., F.R.C.P. Second Edition. 8*s.* 6*d.* net.

The Relief of Pain: A Handbook of Modern Analgesia. By HAROLD BALME M.D., F.R.C.S. Introduction by Sir FARQUHAR BUZZARD, Bt., *K.C.V.O.*, M.D., F.R.C.P. Second Edition. 12*s.* 6*d.* net.

A Clinical Atlas of Blood Diseases. By A. PINEY, M.D., M.R.C.P., and STANLEY WYARD, M.D. Fourth Edition. 42 Illustrations. 38 Coloured. 12*s.* 6*d.*

Also by Dr. A. Piney.

Recent Advances in Hæmatology. Fourth Edition. 8 Coloured Plates. 34 Text-figures. 15*s.* net.

Diseases of the Blood. Second Edition. 65 Illustrations, 14 in Colour. 18*s.* net.

Blood Diseases in Clinical Practice. By Prof. Dr. MORAWITZ. Translated by A. PINEY, M.D. 7*s.* 6*d.* net.

A Textbook of Hematology. By WILLIAM MAGNER, M.D. D.P.H. 29 Illustrations. 18*s.* net.

Taylor's Practice of Medicine. Fifteenth Edition. Revised by Dr. E. P. POULTON and Collaborators. 71 Plates (16 Coloured) and 104 Text-figures. 28*s.* net.

Recent Advances in Endocrinology. By A. T. CAMERON, D.Sc. Third Edition. 65 Illustrations. 15*s.* net.

Tuberculin: Its Vindication by Technique, with Special Reference to Tuberculous Disease of the Eye. By W. CAMAC WILKINSON, M.D., F.R.C.P. 31 Illustrations. 10*s.* 6*d.* net.

Recent Advances in Vaccine and Serum Therapy. By A. FLEMING, M.B., F.R.C.S., and G. F. PETRIE, M.D. 5 Illustrations. 15*s.* net.

Recent Advances in Cardiology. By TERENCE EAST, D.M., F.R.C.P., and C. W. C. BAIN, *M.C.*, D.M. Third Edition. 14 Plates and 85 Text-figures. 12*s.* 6*d.* net.

Recent Advances in the Study of Rheumatism. By F. J. POYNTON, M.D., F.R.C.P., and B. E. SCHLESINGER, M.D., F.R.C.P. Second Edition. 51 Illustrations. 15*s.* net.

Studies in Blood Formation. By T. D. POWER, M.D., D.P.M. 25 Illustrations. 8*s.* 6*d.* net.

Disorders of the Blood. By L. E. H. WHITBY, *C.V.O.*, M.D., and C. J. C. BRITTON, M.D. Third Edition. 12 Plates (8 Coloured) and 61 Text-figures. 21*s.* net.

Modern Treatment of Diseases of the Respiratory System. By A. LISLE PUNCH, M.B., M.R.C.P., and F. A. KNOTT, M.D., M.R.C.P. 96 Plates and 31 Text-figures. 15*s.* net.

Vitamins and Vitamin Deficiencies. By LESLIE HARRIS, D.Sc., Ph.D., Nutritional Laboratory, Medical Research Council and University of Cambridge. To be complete in Seven Volumes. Vol. I. Ready. 50 Illustrations. 8*s.* 6*d.*

Medicine ❧ Massage ❧ Gymnastics

Essentials of Cardiography. By H. B. Russell, M.D., M.R.C.P. 73 Illustrations. 7*s.* 6*d* net.

Recent Advances in Allergy (Asthma, Hayfever, Eczema, Migraine, etc.). By G. W. Bray, M.B., M.R.C.P. Third Edition. 107 Illustrations. 15*s.* net.

Chemical Methods in Clinical Medicine. By G. A. Harrison, M.D., B.Ch. Second Edition. 3 Coloured Plates and 86 Text-figures. 21*s.* net.

Recent Advances in Medicine. Clinical—Laboratory—Therapeutic. By G. E. Beaumont, D.M., F.R.C.P., and E. C. Dodds, *M.V.O.*, M.D., D.Sc. Ninth Edition. 42 Illustrations. 15*s.* net.

Recent Advances in Pulmonary Tuberculosis. By L. S. T. Burrell, M.D. Third Edition. 70 Illustrations. 15*s.* net.

The Diabetic Life: Its Control by Diet and Insulin. By R. D. Lawrence, M.D., Physician in Charge of Diabetic Department, King's College Hospital. Eleventh Edition. 19 Illustrations. 8*s.* 6*d.* net.

The Science and Art of Joint Manipulation. By James Mennell, M.D., B.C. Vol. I.—The Extremities. 284 Illustrations. 18*s.* Vol. II.—The Spinal Column. *In preparation.*

Also by Dr. Mennell.

Physical Treatment by Movement, Manipulation and Massage. Fourth Edition. *Ready Feb., 1940.* **Backache.** Second Edition. 59 Illustrations. 10*s.* 6*d.* net.

Medical Electricity for Massage Students. By Hugh Morris, M.D., D.M.R.E. 103 Illustrations. 15*s.* net.

Massage and Medical Gymnastics. By Mary V. Lace, C.S.M.M.G., Lecturer in the Bedford Physical Training College. 94 Illustrations. 10*s.* 6*d.* net.

A Text-Book of Gymnastics. By K. A. Knudsen, Late Chief Inspector of Gymnastics for Denmark. Translated by F. Braae Hansen. 216 Illustrations. 12*s.* 6*d.* net.

Translated and Edited by Dr. Mina L. Dobbie.

Medical Gymnastics and Massage in General Practice. By Dr. J. Arvedson. Fourth Edition. 8*s.* 6*d.* net.

The Technique, Effects and Uses of Swedish Medical Gymnastics and Massage. By J. Arvedson. Third Edition. 131 Illus. 12*s.* 6*d.* net.

Principles of Gymnastics for Women and Girls. By Elli Björkstén. Part I. 30 Illustrations. 8*s.* 6*d.* net. Part II. 564 Illustrations. 21*s.* net.

Health and Muscular Habits. By Lt.-Col. J. K. McConnel, *D.S.O.*, *M.C.*, and F. W. W. Griffin., M.D. Foreword by Rt. Hon. Lord Horder, *K.C.V.O.*, M.D., F.R.C.P. 27 Illustrations. 5s. net.

Researches on Rheumatism. By F. J. Poynton, M.D., F.R.C.P., and A. Paine, M.D., B.S. 106 Illustrations 15*s.* net.

Chronic Rheumatism, Causation and Treatment. By R. Fortescue Fox, M.D., F.R.C.P., and J. Van Breemen, M.D. 8 Plates and 38 Text-figures. 12*s.* 6*d.* net.

Surgery Radiology

Minor Surgery and the Treatment of Fractures. (Heath, Pollard and Davies). Twenty-second Edition. By Gwynne Williams, M.S., F.R.C.S. 283 Illustrations. 12*s.* 6*d.* net.

Science and Practice of Surgery. By W. H. C. Romanis, F.R.C.S., and P. H. Mitchiner, *C.B.E.*, F.R.C.S. Two vols. Sixth Edition. 800 Illustrations 28*s.* net.

By the same Authors.

Surgical Emergencies in Practice. 158 Illustrations. 18*s.* net.

A Short Textbook of Surgery. By C. F. W. Illingworth, M.D., F.R.C.S.Ed. Second Edition. 12 Plates and 189 Text-figures. 21*s.* net.

Practical X-Ray Therapy. By Hugh Davies, M.R.C.S., D.M.R.E., Officer in Charge of X-Ray Therapy, King's College Hospital. 47 Illustrations. 8*s.* 6*d.* net.

The Radiology of Bones and Joints. By James F. Brailsford, M.D., M.R.C.S. Second Edition. 340 Illus. 30*s.* net.

The M.B., B.S. Finals. A collection of the Papers set at the London M.B., B.S. Examination for the years 1920–1935, classified and arranged in suitable subdivisions. By F. Mitchell Heggs, M.B., B.S., F.R.C.S.Ed. 6*s.* net.

Recent Advances in Radiology. By Peter Kerley, M.D., B.Ch., D.M.R.E. Second Edition. 176 Illustrations. 15*s.* net.

Surgical Radiology. By A. P. Bertwistle, M.B., F.R.C.S.E. 21 Plates. 8*s.* 6*d.* net.

Radiological Terminology. By C. E. Gaitskell, M.R.C.S., L.R.C.P. 5*s.* net.

The After-Treatment of Wounds and Injuries. By R. C. Elmslie, M.S., F.R.C.S. 144 Illustrations. 15*s.* net.

A Textbook of Surgical Pathology. By C. F. W. Illingworth, M.D., F.R.C.S.E., and B. M. Dick, M.B., F.R.C.S.E. Third Edition. 299 Illustrations. 36*s.* net.

Bowlby and Andrewes' Surgical Pathology and Morbid Anatomy. Revised by Geoffrey Keynes, M.D., F.R.C.S. Eighth Edition. With 224 Illustrations. 21*s.* net.

Elementary Surgical Handicraft. By J. Renfrew White, Ch.M., F.R.C.S. 243 Illustrations. 8*s.* 6*d.* net.

Surgical Nursing and After-Treatment. By H. C. Rutherford Darling, M.D., F.R.C.S., Surgeon, Prince Henry Hospital, Sydney. Sixth Edition. With 206 Illustrations. 9*s.* net.

Medical Organisation and Surgical Practice in Air Raids. By P. H. Mitchiner, *C.B.E.*, F.R.C.S., and E. M. Cowell, *C.B.E.*, *D.S.O.*, F.R.C.S. (Reprinted). 51 Illustrations. 10*s.* 6*d.*

Nursing in Time of War. By P. H. Mitchiner, *C.B.E.*, F.R.C.S., and E. E. P. MacManus, *O.B.E.*, S.R.N. 27 Illustrations. 1*s.* 6*d.*

Field Service Notes for Regimental Officers. By E. M. Cowell, *C.B.E.*, *D.S.O.*, F.R.C.S. 29 Illustrations. *Ready Jan., 1940.* Price about 6*s.*

Surgery ❦ Anæsthetics

Surgery of the Hand. By Marc Iselin, M.D., Surgeon, The American Hospital, Paris. Translated by T. M. J. d'Offay, M.B., F.R.C.S. 8 Plates and 120 Text-figures. *Ready Spring, 1940.*

The Operations of Surgery. Eighth Edition. By R. P. Rowlands, M.S., F.R.C.S., and Philip Turner, M.S., F.R.C.S. Volume I. 435 Illustrations, 38 in colour. 36*s.* net. Volume II. 514 Illustrations, 4 in colour. 36*s.* net.

Recent Advances in Orthopædic Surgery. By B. H. Burns, B.Ch., F.R.C.S., and V. H. Ellis, B.Ch., F.R.C.S. 108 Illustrations. 15*s.* net.

Recent Advances in Radium. By W. Roy Ward, M.B., B.S., M.R.C.S., and A. J. Durden Smith, M.B., B.S., M.R.C.S., of The Radium Institute, London. 4 Coloured Plates and 140 black and white Illustrations. 21*s.* net.

Radium Treatment of Cancer. By Stanford Cade, F.R.C.S. With 13 Coloured Plates and 49 Text-figures. 15*s.* net.

The Principles of Radiography. By J. A. Crowther, Sc.D., F.Inst.P. With 55 Illustrations. 7*s.* 6*d.* net.

Recent Advances in Surgery. By W. H. Ogilvie, M.D., F.R.C.S., Assistant Surgeon, Guy's Hospital. Second Edition. 115 Illustrations. 15*s.* net.

Surgery in the Tropics. By Sir Frank P. Connor, *D.S.O.*, F.R.C.S., Professor of Surgery, Bengal Medical College. 99 Illustrations. 12*s.* 6*d.* net.

Operative Surgery of the Head, Neck, Thorax and Abdomen. By Edward H. Taylor, F.R.C.S.I. With 300 Original Illustrations, many in colour. 32*s.* net

Synopsis of Surgery. By Ivor Back, F.R.C.S., and A. T. Edwards, F.R.C.S. 12*s.* 6*d.* net.

Synopsis of Surgical Pathology. By Eric Pearce Gould, M.D., F.R.C.S. 6*s.* net.

Synopsis of Surgical Diagnosis. By W. H. C. Romanis, M.B., M.Ch., F.R.C.S. 8*s.* 6*d.* net.

Practice and Problem in Abdominal Surgery. By Alfred Ernest Maylard, M.B.Lond. and B.S. 39 Illustrations. 8*s.* 6*d.* net. **Abdominal Tuberculosis.** 57 Illustrations. 12*s.* 6*d.* net.

The Truth About Vivisection. By Sir Leonard Rogers, K.C.S.I., LL.D., M.D., F.R.C.S., F.R.S., Hon. Treasurer, Research Defence Society. 9 Illustrations. 5*s.*

Chloroform: a Manual for Students and Practitioners. By Edward Lawrie, M.B.Edin. Illustrated. 7*s.* 6*d.* net.

Recent Advances in Anæsthesia and Analgesia. By C. Langton Hewer, M.B., B.S., D.A.(R.C.P.& S.). Third Edition. 132 Illustrations. 15*s.* net.

Practical Points in Anæsthesia. By H. K. Ashworth, M.B., Ch.B., D.A.(R.C.P. & S.), Anæsthetist to the Royal Infirmary, Manchester. 16 Illustrations. 7*s.* 6*d.* net.

Dermatology ⁂ Urinary Disorders
Neurology ⁂ Tropical Diseases

An Introduction to Dermatology. By E. H. Molesworth, M.D., Ch.M. Foreword by Prof. Josef Jadassohn. 151 Illustrations. 25*s.* net.

Recent Advances in Dermatology. By W. Noel Goldsmith, M.D., M.R.C.P. 8 Coloured Plates and 50 Text-figures. 18*s.* net.

A Text-Book of Diseases of the Skin. By J. H. Sequeira, M.D., F.R.C.P., F.R.C.S. 4th Edition. 56 Plates in colours and 309 Text-figures. 42*s.* net.

Skin: Its Uses in Six Phases. By Lewis E. Hertslet, M.R.C.S., L.R.C.P. 8 Plates. 10s. 6d. net.

The Hair, its Care, Diseases and Treatment. By W. J. O'Donovan, *O.B.E.*, M.D., B.S., Physician, Skin Dept., London Hospital. 40 Illustrations. 12*s.* 6*d.* net.

Recent Advances in Neurology. Fourth Edition. By W. Russell Brain, D.M., F.R.C.P. 24 Illustrations. 15*s.* net.

Diseases of the Kidney. By Sir Girling Ball, F.R.C.S., and Geoffrey Evans, M.D., F.R.C.P. 8 Colour Plates and 159 Illustrations. 36*s.* net.

Kidney Pain: Its Causation and Treatment. By G. Leon Jona, M.D., M.S., F.R.A.C.S. 61 Illustrations. 7*s.* 6*d.* net.

Stone in the Urinary Tract. By H. P. Winsbury-White, M.B., Ch.B., F.R.C.S. 2 Col. Plates and 181 Text-figs. 25*s.*

Recent Advances in Genito-Urinary Surgery. By Hamilton Bailey, F.R.C.S., and Norman Matheson, M.B., F.R.C.S. 89 Illustrations. 15*s.* net.

The Nematode Parasites of Vertebrates. By Warrington Yorke, M.D., and P. A. Maplestone, *D.S.O.*, M.B. Foreword by C. W. Stiles. 307 Illustrations. 36*s.* net.

Recent Advances in Tropical Medicine. By Sir Leonard Rogers, *K.C.S.I.*, M.D., F.R.S., F.R.C.S., F.R.C.P. Second Edition. 16 Illustrations. 12*s.* 6*d.* net.

Public Health Practice in the Tropics. By J. Balfour Kirk, M.B., D.P.H., D.T.M.&H., Director, Medical and Health Department, Mauritius. 80 Illustrations. 15*s.* net.

Tropical Medicine. By Sir Leonard Rogers, *K.C.S.I.*, F.R.C.P., F.R.C.S., F.R.S., and Sir J. W. D. Megaw, *K.C.I.E.*, M.B., B.Ch. Third Edition. 2 Colour Plates and 82 Illustrations. 16*s.* net.

The Seasonal Periodicity of Malaria and the Mechanism of the Epidemic Wave. By C. A. Gill, Col. I.M.S. (Ret.), M.R.C.P., D.T.M. & H. Map and 17 Illustrations. 10*s.* 6*d.* net.

Malarial Nephritis. By George Giglioli, M.D. (Italy), D.T.M.&H.(Eng.). 17 Illustrations. 8*s.* 6*d.* net.

The Health Game. A Popular Guide for the Tropics. By B. E. Washburn, M.D. 20 Illustrations. 5*s.* net.

J. & A. CHURCHILL Ltd.

Medical Jurisprudence and Toxicology ❧ Ophthalmology

Recent Advances in Forensic Medicine. By Sydney Smith, M.D., D.P.H., and J. Glaister, M.D., Ch.B. Second Edition. 85 Illustrations. 15*s*. net.

Poisons: Their Isolation and Identification. By Frank Bamford, B.Sc. 21 Illustrations. 18*s*. net.

Clinical Toxicology. By E. Leschke. Translated by C. P. Stewart, M.Sc., Ph.D., and O. Dorrer, Ph.D. 25 Illus. 15*s*. net.

Medical Aspects of Crime. By W. Norwood East, M.D., F.R.C.P., H.M. Commissioner of Prisons. 18 Illus. 18*s*. net.

Medical Jurisprudence: its Principles and Practice. By Alfred S. Taylor, M.D. Ninth Edition, by Sydney Smith, M.D., D.P.H., and W. G. H. Cook, LL.D., Barrister-at-Law. 2 vols. 63*s*. net.

An Introduction to Forensic Psychiatry in the Criminal Courts. By W. N. East, M.D. 16*s*. net.

Forensic Medicine. By Sydney Smith, M.D., D.P.H. Sixth Edition. 169 Illustrations. 24*s*. net.

Forensic Medicine. Illustrated by Photographs and Descriptive Cases. By H. Littlejohn, F.R.C.S.Ed. 183 Illus. 15*s*. net.

The Medico-Legal Post-Mortem in India. By D. P. Lambert, M.D., Ch.B., D.T.M.&H. 5*s*. net.

Recent Advances in Ophthalmology. By Sir W. Stewart Duke-Elder, M.D., F.R.C.S. Third Edition. Plates (2 Coloured) and 150 Text-figs. 15*s*. net.

Also by Sir Stewart Duke-Elder.

The Practice of Refraction. Third Edition. 183 Illus. 12*s*. 6*d*. net.

A Handbook of Ophthalmology. By Humphrey Neame, F.R.C.S., and F. A. Williamson-Noble, F.R.C.S. Third Edition. 12 Coloured Plates. 143 Illustrations. 13*s*. 6*d*. net.

Refraction of the Eye, including Physiological Optics. By Charles Goulden, *O.B.E.*, M.D., F.R.C.S. Second Edition. 181 Illustrations. 12*s*. 6*d*. net.

Diseases of the Eye: a Manual for Students and Practitioners. By Sir J. H. Parsons, *C.B.E.*, D.Sc., F.R.C.S., F.R.S. Ninth Edition. 21 Plates and 360 Text-figures. 18*s*. net.
Elementary Ophthalmic Optics, including Ophthalmoscopy and Retinoscopy. 66 Illustrations. 6*s*. 6*d*. net.

Sight-Testing Made Easy, including Chapter on Retinoscopy. By W. W. Hardwicke, M.D. Fourth Edition. 5*s*. net.

Ophthalmological Society of the United Kingdom. Transactions. Annually. 30*s*. net.

The Slit-Lamp Microscopy of the Living Eye. By F. Ed. Koby. Translated by C. B. Goulden, *O.B.E.*, M.D., F.R.C.S., and Clara L. Harris, M.B., Ch.B. Second Edition. 104 Illus. 15*s*. net.

Ophthalmic Nursing. By M. H. Whiting, *O.B.E.*, F.R.C.S. Second Edition. 54 Illustrations. 5*s*. net.

An Atlas of External Diseases of the Eye. By Humphrey Neame, F.R.C.S. 51 Coloured Illustrations. 15*s*. net.

Midwifery   Gynæcology

Text-book of Gynæcology. By WILFRED SHAW, M.D., F.R.C.S.. Physician Accoucheur, with Charge of Out-Patients, St. Bartholomew's Hospital. Second Edition. 4 Coloured Plates and 253 Text-figures. 18*s.* net.

Eden and Lockyer's Gynæcology. Fourth Edition. Revised and re-written by Sir BECKWITH WHITEHOUSE, M.B., M.S., F.R.C.S. 36 Coloured Plates and 619 Text-figures. 38*s.* net.

Antenatal and Postnatal Care. By F. J. BROWNE, M.D., Ch.B., F.R.C.S.E. Third Edition. 101 Illustrations. 21*s.* net.

A Short Textbook of Midwifery. By G. F. GIBBERD, F.R.C.S., M.R.C.O.G., Assistant Obstetric Surgeon, Guy's Hospital. 187 Illustrations. 15*s.* net.

The Queen Charlotte's Text-book of Obstetrics. By Members of the Staff of the Hospital. Fifth Edition. 4 Coloured Plates and 293 Text-figures. 21*s.* net.

Recent Advances in Obstetrics and Gynæcology. By ALECK W. BOURNE, F.R.C.S., and LESLIE WILLIAMS, M.D., F.R.C.S., Obstetric Surgeons, St. Mary's Hospital. Fourth Edition. 98 Illustrations. 15*s.* net.

Handbook of Midwifery. By R. E. TOTTENHAM, M.D., B.A.O., F.R.C.P.I. 102 Illustrations. 10*s.* 6*d.* net.

The Difficulties and Emergencies of Obstetric Practice. By Sir COMYNS BERKELEY, M.D., F.R.C.P., and VICTOR BONNEY, M.D., F.R.C.S. Third Edition. 309 Illustrations. 36*s.* net.

Manual of Obstetrics (EDEN AND HOLLAND.) Eighth Edition. By EARDLEY HOLLAND M.D., F.R.C.P., F.R.C.S. 12 Plates (5 coloured) and 398 Text-figures. 24*s.* net.

A Short Practice of Midwifery. By HENRY JELLETT, M.D., B.A.O.Dub. Tenth Edition. 3 Coloured Plates and 283 Illustrations. 18*s.* net. **A Short Practice of Midwifery for Nurses,** with a Glossary of Medical Terms, and the Regulations of the C.M.B. Tenth Edition. 7 Plates and 183 Illustrations. 8*s.* 6*d.* net. **A Short Practice of Gynæcology.** Revised in collaboration with Professor R. E. TOTTENHAM, M.D., F.R.C.P.I. Sixth Edition. With 365 Illustrations (many in colour). 21*s.* net. **The Causes and Prevention of Maternal Mortality** 15*s.* net.

A Manual for Midwives. By J. B. BANISTER, M.D., F.R.C.S.E. Fourth Edition. 53 Illustrations. 6*s.* net.

Cæsarean Section. By FRANCES IVENS-KNOWLES, *C.B.E.*, M.B., M.S. 5*s.* net.

Practical Talks to Midwives. By E. M. DOUBLEDAY, S.R.N., Sister Tutor, Post Certificate School for Midwives, Camberwell. 17 Illustrations. 3*s.* 6*d.* net.

Midwifery for Nurses. By A. W. BOURNE, M.B., F.R.C.S. Second Edition. 110 Illustrations. 6*s.* 6*d.* net.

Obstetric Regulations for Use in Obstetric Units. By G. W. THEOBALD, M.D., F.R.C.S.Ed. 1*s.* 6*d.* net.

J. & A. CHURCHILL Ltd.

Pædiatrics · Otology · Dentistry

The Care of Young Babies. By John Gibbens, M.B., M.R.C.P. With a Foreword by Sir Robert Hutchison, Bt., M.D., F.R.C.P. 8 Plates and 5 Text-figures. 3*s*. 6*d*. net.

Training for Childbirth: From the Mother's Point of View. By Minnie Randell, S.R.N., S.C.M., T.M.M.G., Principal, School of Massage, and Sister-in Charge, Dept. of Physiotherapy, St. Thomas's Hospital. 118 Illustrations, some in colour. 7*s*. 6*d*. net.

Mothercraft: Antenatal and Postnatal. By R. C. Jewesbury, D.M., F.R.C.P. 2nd Edition. 21 Illus., 13 in Colour. 10*s*. 6*d*.

Diseases of Infancy and Childhood. By Wilfrid Sheldon, M.D., F.R.C.P. Second Edition. 13 Plates and 125 Text-figures. 21*s*. net.

Recent Advances in Diseases of Children. By W. J. Pearson, *D.S.O.*, D.M., F.R.C.P., and W. G. Wyllie, M.D., M.R.C.P. Third Edition. 23 Plates and 38 Text-figures. 15*s*. net.

The Mothercraft Manual. By M. Liddiard, S.R.N., Nursing Director, Mothercraft Training Society. 9th Edition. 10 Plates and 32 Text-figures. 3*s*. 6*d*. net.

Recent Advances in Laryngology and Otology. By R. Scott Stevenson, M.D., F.R.C.S.Ed. 128 Illustrations. 15*s*. net.

Manual of Diseases of Nose and Throat. By C. G. Coakley, M.D. Seventh Edition. 153 Illus. and 7 Coloured Plates. 18*s*. net.

The Pharmacopœia of the Golden Square Throat, Nose and Ear Hospital. Eighth Edition. 2*s*. 6*d*. net.

Oral Diagnosis and Treatment Planning. By S. C. Miller, D.D.S. (Editor), and Twenty-Two Specialist Contributors. 562 Illustrations. 30*s*. net.

An Introduction to Dental Anatomy and Physiology, Descriptive and Applied. By A. Hopewell-Smith, L.D.S.Eng. With 6 Plates and 340 Illustrations 21*s*. net.

Tomes' Dental Anatomy, Human & Comparative. Edited by H. W. Marett Tims, *O.B.E.*, M.A., M.D., F.Z.S., and C. Bowdler Henry, L.R.C.P., M.R.C.S., L.D.S.Eng. Eighth Edition. 325 Illustrations. 18*s*. net.

Tomes' System of Dental Surgery. Fifth Edition. Revised by Sir Charles Tomes, F.R.S., and Walter S. Nowell, M.A. Oxon. 318 Engravings. 15*s*. net.

A Manual on Dental Metallurgy. By Ernest A. Smith, Assoc., Roy. School of Mines. Fifth Edition. 15 Illus. 12*s*. 6*d*. net.

Handbook of Mechanical Dentistry. By J. L. Dudley Buxton, L.D.S. With 168 Illustrations. 12*s*. 6*d*. net.

Operative Dentistry. By W. H. O. McGehee, M.D., D.D.S. Second Edition. 1040 Illustrations. 42*s*. net.

A Dictionary of Dental Science and Art. 79 Illustrations. 28*s*. net. (See page 5.)

Chemistry

Poisons: Their Isolation and Identification. By Frank Bamford, B.Sc. 21 Illustrations. 18*s*. net.

The War Gases—Chemistry and Analysis. By Dr. Mario Sartori, Chemist of the Italian Chemical Warfare Service. Translated from the Second, Enlarged, Italian Edition by L. W. Marrison, B.Sc., A.I.C. 20 Illustrations. 21*s*. net.

The Plant Alkaloids. By T. A. Henry, D.Sc. 3rd Edition. 42*s*. net.

The Chemical Analysis of Foods. By H. E. Cox, D.Sc., Ph.D. Second Edition. 41 Illustrations. 21*s*. net.

Colloid Aspects of Food Chemistry. By W. Clayton, D.Sc., F.I.C. 64 Illustrations. 36*s*. net.

The Chemistry, Flavouring and Manufacture of Chocolate Confectionery and Cocoa. By H. R. Jensen, M.Sc., F.I.C. 23 Illustrations. 27*s*. net.

A Chemical Dictionary: Containing the Words generally used in Chemistry and many of the Terms used in the Related Sciences. By Ingo W. D. Hackh, A.M., F.A.I.C., and Julius Grant, M.Sc., Ph.D. Many Tables and Illustrations. 48*s*. net.

Modern Methods of Cocoa and Chocolate Manufacture. By H. W. Bywaters, D.Sc., Ph.D., A.R.C.S., F.I.C. 108 Illustrations. 21*s*. net.

Gasworks Laboratory Handbook. By W. I. Ineson, Chief Chemist, Bradford Corporation Gasworks. 55 Illustrations. 9*s*. 6*d*. net.

Organic Medicaments and their Preparation. By E. Fourneau. Translated by W. A. Silvester, M.Sc. 22 Illustrations. 15*s*. net.

The Fundamental Processes of Dye Chemistry. By H. E. Fierz-David. Translated by F. A. Mason, Ph.D. 45 Illus. including 19 Plates. 21*s*. net.

A Junior Inorganic Chemistry. By R. H. Spear, M.A. Second Edition. 97 Illustrations. 6*s*. 6*d*. Also **Part I** (up to Atomic Theory). 3*s*. 6*d*. net.

Explosives. Their Manufacture, Properties, Tests, and History. By A. Marshall, A.C.G.I. Second Edition. 3 vols. 158 Illustrations. Vols. I and II, £3 3*s*. net. Vol. III, £2 2*s*. **A Short Account of Explosives.** 7*s*. 6*d*. net. **A Dictionary of Explosives.** 15*s*. net.

Inorganic and Organic Chemistry. By C. L. Bloxam. Eleventh Edition. By A. G. Bloxam, F.I.C., and S. Judd Lewis, D.Sc., F.I.C. 36*s*. net.

Treatise on Applied Analytical Chemistry. Edited by Prof. V. Villavecchia. Translated by T. H. Pope, B.Sc. Vol. I. With 58 Illustrations. 21*s*. net. Vol. II. With 105 Illustrations. 25*s*. net.

Treatise on General and Industrial Chemistry. By Dr. Ettore Molinari. Second English Edition. Translated by T. H. Pope, B.Sc., F.I.C. Vol. I.—**Inorganic.** 328 Illus., 42*s*. net. Vol. II.—**Organic.** Part I. 254 Illus., 30*s*. net. Part II. 303 Illus., 30*s*. net.

Ammonia and the Nitrides. By E. B. Maxted, Ph.D., D.Sc. 7*s*. 6*d*. net.

Industrial Organic Analysis. By Paul S. Arup, B.Sc., A.C.G.I. Second Edition. 25 Illustrations. 12*s*. 6*d*. net.

Reagents and Reactions. By E. Tognoli. Trans. by C. A. Mitchell, D.Sc. 7*s*. 6*d*. net.

Laboratory Manual of Elementary Colloid Chemistry. By E. Hatschek. Second Edition. With 21 Illustrations. 7*s*. 6*d*. net.

The Atmospheric Nitrogen Industry. By Dr. I. B. Waeser. Translated by E. Fyleman, Ph.D. 2 Vols. 72 Illustrations. 42*s*. net.

Chemistry

Chemistry of the Proteins. By DOROTHY JORDAN LLOYD, D.Sc., F.I.C., and AGNES SHORE, B.Sc., A.I.C. Second Edition. 101 Illustrations. 21*s*. net.

Pregl's Quantitative Organic Microanalysis. By Dr. HUBERT ROTH, Third English Edition. Translated by E. BERYL DAW, B.Sc., A.I.C. 72 Illustrations. 18*s*. net.

The Theory of Emulsions and their Technical Treatment. By W. CLAYTON, D.Sc., F.I.C. Third Edition. 91 Illustrations. 25*s*. net.

The Natural Organic Tannins: History; Chemistry; Distribution. By M. NIERENSTEIN, D.Sc. Numerous Tables. 21*s*. net.

Adulteration and Analysis of Foods and Drugs. By J. F. LIVERSEEGE, F.I.C., Ph.C., Formerly Public Analyst to the City of Birmingham. Foreword by the Rt. Hon. NEVILLE CHAMBERLAIN, M.P. 36*s*. net.

Recent Advances in Physical Chemistry. By SAMUEL GLASSTONE, D.Sc., Ph.D. Third Edition. 31 Illustrations. 15*s*. net.

Recent Advances in General Chemistry. By S. GLASSTONE, D.Sc., Ph.D. 25 Illustrations. 15*s*. net.

Catalysis and Its Industrial Applications. By E. B. MAXTED, D.Sc., Ph.D., F.I.C. 225 Tables and 66 Illustrations. 36*s*. net.

Theoretical Organic Chemistry. By FRANCIS ARNALL, Ph.D., M.Sc., and FRANCIS W. HODGES, M.Sc. Part I. 30 Illustrations. 115 Experiments. 10*s*. 6*d*. net. Part II. 12*s*. 6*d*. net.

Oils, Fats and Fatty Foods. By E. RICHARDS BOLTON F.I.C. Second Edition. 12 Plates and 34 Text-figures. 30*s*. net.

Sutton's Systematic Handbook of Volumetric Analysis. 12th Edition, by A. D. MITCHELL, D Sc., F.I.C. 128 Illustrations. 35*s*. net.

A Text-Book of Practical Chemistry. By G. F. HOOD, M.A., B.Sc., and J. A. CARPENTER, M A. 162 Illus. 21*s*. net.

Introduction to Qualitative Chemical Analysis. By C. R. FRESENIUS. 17th Edition, translated by C. A. MITCHELL, D.Sc. 57 Illustrations. 36*s*. net.

The Analyst's Laboratory Companion. By A. E. JOHNSON, B.Sc., F.I.C. Fifth Edition. 10*s*. 6*d*. net.

Allen's Commercial Organic Analysis: Fifth Edition, in 10 vols. Edited by C. A. MITCHELL, D.Sc., M.A., S. S. SADTLER, S.B., and C. E. LATHROP, A.B., Ph.D. 32*s*. net each volume.

Volumetric Analysis for Students of Pharm. and General Chemistry By C. H. HAMPSHIRE, M.B., B.S., B.Sc., F.I.C. Fifth Edition. 8*s*. 6*d*. net.

Quantitative Chemical Analysis (Clowes & Coleman). Fourteenth Edition. Revised by D. STOCKDALE, Ph.D., A.I.C., and J. DEXTER, B.Sc., A.I.C. 130 Illustrations. 18*s*. net.

Elementary Practical Chemistry. By F. CLOWES, D.Sc., and J. B. COLEMAN, A.R.C.Sci. Part I. General Chemistry. 7th Ed. 76 Illustrations. 6*s*. net.

Elementary Analytical Chemistry (CLOWES and COLEMAN). 13th Edition revised by G. C. LYONS. M.A., Ph.D., and F. N. APPLEYARD, F.I.C., Ph.C. 15 Illustrations. 7*s*. 6*d*. net.

An Elementary Text-Book of General Microbology. By WARD GILTNER, 99 Illustrations. 15*s*. net.

Clouds and Smokes. The Properties of Disperse Systems in Gases. By W. E. GIBBS, D.Sc. 30 Illustrations. 10*s*. 6*d*. net.

Catalytic Hydrogenation and Reduction. By E. B. MAXTED, Ph.D., D.Sc., With 12 Illustrations. 5*s*. net.

Molecular Physics and the Electrical Theory of Matter. By J. A. CROWTHER, Sc.D. Fourth Edition. With 33 Illustrations. 7*s*. 6*d*. net.

Notes on Chemical Research. By W. P. DREAPER, O.B.E., F.I.C. Second Edition. 7*s*. 6*d*. net.

An Introduction to the Physics and Chemistry of Colloids. By EMIL HATSCHEK. Fifth Edition. With 22 Illustrations. 7*s*. 6*d*. net.

Catalysis and its Industrial Applications. By E. JOBLING, M.C., A.R.C.Sc., B.Sc. Second Edition. 12 Illustrations. 7*s*. 6*d*. net.

Physics ❧ Microscopy ❧ Biology

Recent Advances in Atomic Physics. By CASTELFRANCHI. Translated by W. S. STILES, Ph.D., and J. W. T. WALSH, M.A., D.Sc. 2 Vols. 190 Illustrations. 15*s*. net. each vol.

Recent Advances in Physics (Non-Atomic). By F. H. NEWMAN, D.Sc. 51 Illustrations. 15*s*. net.

The Physics of X-Ray Therapy. By W. V. MAYNEORD, M.Sc. 106 Illustrations. 10*s*. 6*d*. net.

Elementary Physics. By G. STEAD, M.A. Fifth Edition (Enlarged). 430 Illustrations. 12*s*. 6*d*. net.

Recent Advances in Microscopy. Edited by A. PINEY, M.D., M.R.C.P. 83 Illustrations. 12*s* 6*d*. net.

Elementary Histological Technique for Animal and Plant Tissues. By J. T HOLDER, F.R.M.S. 23 Illustrations. 7*s*. 6*d*. net.

The Microtomist's Vade-Mecum (BOLLES LEE). Tenth Edition. Edited by J. BRONTË GATENBY, D.Sc , and T. S. PAINTER, A.M., Ph.D. 11 Illustrations. 30*s*. net.

A Text-Book of Botany, for Medical, Pharmaceutical and Other Students. By J. SMALL, D.Sc., F.L.S. Fourth Edition. 1350 Illustrations. 21*s*. net. **Practical Botany.** 35 Illustrations. 10*s*. 6*d*. net. **Pocket Lens Plant Lore.** 25 Illustrations. 5*s*. net.

Plant Physiology. By MEIRION THOMAS, M.A. Reprinted with Additions. 57 Illustrations. 15*s* net.

Recent Advances in Plant Physiology. By E. C. BARTON-WRIGHT, M.Sc. Second Edition. 54 Illustrations. 12*s*. 6*d*. net.
Recent Advances in Botany. 60 Illustrations. 12*s*. 6*d*. net.

Recent Advances in Entomology. By A. D. IMMS, D.Sc., F.R.S. Second Edition. 94 Illustrations. 15*s*. net.

Recent Advances in Plant Genetics (SANSOME AND PHILP). Second Edition. By F. W. SANSOME, Ph.D., F.R.S.E. 55 Illustrations and 49 Tables. 18*s*. net.

Recent Advances in Cytology. By C. D. DARLINGTON, D Sc., Ph.D. Second Edition. 16 Plates and 160 Text-figures. 21*s*. net.

Recent Advances in Agricultural Plant Breeding. By H. HUNTER, D.Sc., and H. M. LEAKE, Sc.D. 16 Plates. 15*s*. net.

Recent Advances in the Study of Plant Viruses. By KENNETH SMITH, D.Sc., Ph.D. 1 Col. Plate and 67 Illus. 15*s*. net.

ALSO

A Text-book of Plant Virus Diseases. 101 Illustrations. 21*s*. net.

An Introduction to Comparative Zoology. By F. G. SAREL WHITFIELD, F.R.E.S., F.R.M.S., and A. H. WOOD, M.A. 141 Illustrations. 15*s*. net.

Biological Laboratory Technique: An Introduction to Research in Embryology, Cytology and Histology. By J. BRONTË GATENBY, M.A., Ph.D., D.Sc. 8 Illustrations. 7*s*. 6*d*. net.

J. & A. CHURCHILL Ltd., 104 GLOUCESTER PLACE, W.